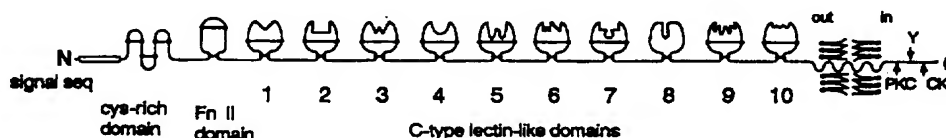




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(54) Title: IDENTIFICATION OF DEC, (DENDRITIC AND EPITHELIAL CELLS, 205 kDa), A RECEPTOR WITH C-TYPE LECTIN DOMAINS, NUCLEIC ACIDS ENCODING DEC, AND USES THEREOF			



(57) Abstract

The present invention relates to the identification and characterization of a receptor associated with antigen presentation in immune responses, endocytosis, and transepithelial transport. Identification of the receptor, its characterization as having ten lectin-binding domains, and evidence of its role in the uptake and processing of oligosaccharides and oligosaccharide-decorated molecules, e.g. glycoproteins, has important ramifications for modifying immune response, and for trans-epithelial transport of molecules. The receptor, or integral membrane protein, termed herein "DEC", is found primarily on dendritic cells, but also found on B cells, brain capillaries, bone marrow stroma, epithelia of intestinal villi and pulmonary airways, as well as cortical epithelium of the thymus and the dendritic cells in the T cell areas of peripheral lymphoid organs. The murine and human counterparts of DEC have an apparent molecular mass of 205 kDa, the murine counterpart has an isoelectric point at pH 7.5, and carbohydrates comprise about 7 kDa of the total mass of murine DEC. The present invention is directed to identification of additional ligands of DEC, which can be advantageously targeted to dendritic cells and other cells that bear DEC. Targeting antigens for presentation by dendritic cells can provide for tolerance when the dendritic cells are quiescent, or for immune stimulation (i.e., vaccination) when the dendritic cells are activated, e.g., by stimulation with a cytokine or lymphokine, such as colony stimulating factor (CSF).

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IDENTIFICATION OF DEC, (Dendritic and Epithelial Cells, 205kDa),
A RECEPTOR WITH C-TYPE DOMAINS, NUCLEIC ACIDS ENCODING DEC,
AND USES THEREOF

The research leading to the present invention was supported in part with National
5 Institutes of Health Grant No. AI13013. The Government may have certain rights
in the invention.

FIELD OF THE INVENTION

10 The present invention relates to the identification and characterization of a receptor
associated with antigen presentation in immune responses, endocytosis, and trans-
epithelial transport. Identification of the receptor, its characterization as having
ten lectin-binding domains, and evidence of its role in the uptake and processing of
oligosaccharides and oligosaccharide-decorated molecules, *e.g.*, glycoproteins, has
15 important ramifications for modifying immune response, and for trans-epithelial
transport of molecules.

BACKGROUND OF THE INVENTION

20 Dendritic cells are a unique class of leukocytes whose primary function is to
capture, process, and present antigens to T cells (Steinman, 1991, Annu. Rev.
Immunol. 9:271-96). Interaction between dendritic cells and specific T cells in the
peripheral immune system leads to the induction of immune responses, whereas in
the thymus presentation by dendritic cells leads to negative selection (Tanaka et
25 al., 1993, Eur. J. Immunol. 23:2614-2621; Matzinger et al., 1989, Nature 338:74-
76). Like dendritic cells, thymic epithelial cells present MHC-bound peptides to T
cells, but instead of inducing T cell activation or negative selection, thymic
epithelial cells direct positive selection (Hugo et al., 1993, Immunol. Rev.
135:133-35; Elliott, Immunol. Rev. 135:215-25). Consistent with the known
30 requirements for interactions with T cells, both dendritic cells and thymic
epithelial cells express a number of cell surface proteins that facilitate cell-cell
contact and mediate T cell activation (Steinman, 1991, Annu. Rev Immunol.
9:271-96; Hugo et al., 1993, Immunol Rev. 135:133-35; Elliott, Immunol Rev.

135:215-25). An additional fundamental requirement for both dendritic cells and thymic epithelial cells is the uptake and processing of antigen, yet neither cell type is known to express receptors that are specialized for antigen capture or presentation.

5

Over the decade since its initial isolation by Kraal et al. (J. Exp. Med. 163:981), the monoclonal antibody NLDC-145 has been utilized as a histochemical and flow cytometric marker for mouse dendritic cells (DCs) in a variety of tissues (Kraal et al., *supra*; Crowley et al., 1989, Cell Immunol. 118:108; Vremec et al., 1992, J. Exp. Med. 176:47; Pollard and Lipscomb, 1990, J. Exp. Med. 172:159; Soesatyo et al., 1990, Cell Tiss. Res. 259:587; Austyn et al., 1994, J. Immunol. 152:2401; Lu et al., 1994, J. Exp. Med. 179:1823; and Breel et al., 1988, Immunol. 63:657). The antigen bound by NLDC-145 is also abundant on thymic cortical epithelium. However, cloning and characterization of the NLDC-145 antigen has
10 proved elusive. For one thing, dendritic cell cDNA libraries have not been readily prepared. Dendritic cells themselves are rare, making their RNA extremely rare. Moreover, monoclonal antibodies are not usually effective reagents for screening expression libraries, *e.g.*, a λ gt-11 expression library.

15 Accordingly, there is a need in the art to clone and characterize the antigen recognized by monoclonal antibody NLDC-145.

There is a further need in the art to harness the immunomodulatory abilities of dendritic cells, *e.g.*, to induce tolerance or immunity.

25

The citation of any reference herein should not be construed as an admission that such reference is prior art to the instant invention.

SUMMARY OF THE INVENTION

The present invention relates to an integral membrane protein, termed herein "DEC," found primarily on dendritic cells, but also found on B cells, brain capillaries, bone marrow stroma, epithelia of intestinal villi and pulmonary airways, as well as cortical epithelium of the thymus and the dendritic cells in the T cell areas of peripheral lymphoid organs. In addition, trace amounts of this protein are found in organs like the liver, heart, and kidney. The murine and human counterparts of DEC have an apparent molecular mass of 205 kDa, the murine counterpart has an isoelectric point at pH 7.5, and carbohydrates comprise about 7 kDa of the total mass of murine DEC. The carbohydrates appear to consist of eight distinct but related biantennary N-linked glycans, with no O-linked glycans present. Because the protein has been found predominantly on Dendritic cells and thymic Epithelial Cells, and has a molecular weight of 205 kDa, it is termed DEC-205. In a specific embodiment, the invention relates to isolation and cloning of human DEC, which is further characterized by having a carboxyl-terminal sequence RHRLHLAGFSSVRYAQGVNEDEIMLP SFHD (SEQ ID NO: 1), and characterized by binding to a rabbit polyclonal antibody raised against full length murine DEC-205, but not reacting with monoclonal antibody NLDC-145. In a more specific embodiment, human DEC has the amino acid sequence depicted in SEQ ID NO:8; it may be encoded by the nucleotide sequence depicted in SEQ ID NO:7. Another characteristic of DEC, based on the deduced amino acid sequence information obtained from cloning the *dec* cDNA is a unique coated pit localization consensus domain or motif on the cytoplasmic tail of the protein, which, as shown in Figure 9, has regions of homology and regions of dissimilarity between the two counterpart proteins. It has been further discovered that DEC-205 is rapidly internalized via coated vesicles, and delivers bound substances to a multivesicular endosomal compartment that resembles the MHC class-II containing vesicles implicated in antigen processing.

Accordingly, in its primary aspect, the present invention is directed to identification of additional ligands of the DEC-205 receptor, which can be advantageously targeted to dendritic cells and other cells that bear DEC-205. Targeting antigens for presentation by dendritic cells can provide for tolerance
5 when the dendritic cells are quiescent, or for immune stimulation (*i.e.*, vaccination) when the dendritic cells are activated, *e.g.*, by stimulation with a cytokine or lymphokine, such as colony stimulating factor (CSF).

Furthermore, the presence of DEC on epithelial cells suggests an important role in
10 trans-epithelial transport of molecules, *e.g.*, from the basolateral surface of the lung respiratory epithelium into lung airways or the basolateral surface of the intestinal epithelium into the lumen of the intestines, and from the apical (luminal) surface of these epithelial to the pulmonary or intestinal circulation, respectively. Thus, the invention provides for parenteral delivery of pharmacological agents,
15 *e.g.*, antibiotics, to infections of the lung or the intestines, by targeting the pharmacological agent with a ligand to DEC, and for systemic delivery of pharmacological agents by aerosolization and inhalation via the lungs, or ingestion and absorption via the intestines.

20 In a further aspect, the invention provides for targeting pharmacological agents to cross the blood brain barrier via DEC located on the brain capillaries.

Thus, in a preferred aspect, the invention provides a method for identifying a ligand for DEC, comprising contacting a protein comprising at least one DEC
25 lectin domain with a candidate ligand; and detecting binding of the candidate ligand with the DEC lectin domain. Binding of the candidate ligand and the DEC lectin domain indicates that the ligand candidate is a ligand for DEC. In a preferred aspect, the ligand is a saccharide, which binds to one or more of the lectin domains on DEC.

According to one aspect of the invention, the protein comprising at least one DEC lectin domain is expressed by cells as an integral membrane protein, and the candidate ligand is labeled, such that binding of the candidate ligand with the DEC lectin domain is detected by detecting association of the label with the cells. In another embodiment, the protein comprising at least one DEC lectin domain is solubilized, and the candidate ligand is irreversibly associated with a solid phase support, such that binding of the candidate ligand with the DEC lectin domain is detected by detecting binding of the protein with the solid phase support. In yet another embodiment, the protein comprising at least one DEC lectin domain is irreversibly associated with a solid phase support, and the candidate ligand is labeled, such that binding of the candidate ligand with the DEC lectin domain is detected by detecting association of label with the solid phase support. In one embodiment, the protein comprising at least one DEC lectin domain is a truncated DEC protein; in another embodiment, the protein comprising at least one DEC lectin domain is a full length DEC protein.

The present invention advantageously provides a nucleic acid encoding at least a portion of a DEC protein. Thus, the invention provides for expression of DEC proteins, or truncated fragments thereof, including chimeric proteins, which can be used for identifying a DEC ligand. Moreover, the nucleic acid of the invention comprises at least fifteen base pairs, thus, the nucleic acids of the invention provide useful probes for detecting expression of mRNA for DEC, PCR primers for reverse transcriptase polymerase chain reaction (RT-PCR) amplification of RNA, or for cloning DEC, and probes for the presence of DEC cDNA or genomic DNA, *e.g.*, in a library or cell. In a preferred embodiment, the nucleic acid encodes a human DEC protein. In a specific embodiment, *infra*, a nucleic acid encoding human DEC is provided.

The present invention further provides an expression vector comprising the nucleic acid encoding DEC, wherein the nucleic acid is a DNA molecule encoding at least a lectin domain of DEC, operatively associated with an expression control

sequence. In a further aspect, the invention provides a recombinant host cell comprising the expression vector. In various embodiments, the host cell is a mammalian cell selected from the group consisting of a Chinese hamster ovary cell, an African Green Monkey COS cell, a Madin-Darby canine kidney cell, and
5 an NIH-3T3 fibroblast cell.

The invention further provides an antibody reactive with a human DEC-205 protein, in particular a monoclonal antibody and a polyclonal antibody.

10 As mentioned above, the present invention advantageously provides for identifying ligands of DEC, which ligands are capable of targeting a molecule to which they are attached, *i.e.*, conjugated, to a cell bearing DEC *in vitro* or *in vivo*. The ability to target cells that express DEC *in vivo* has important implications from the perspective of specifically targeting dendritic cells, epithelial cells, *e.g.*, of the
15 thymus, small intestine, and lung. Thus, the invention is naturally directed to a pharmaceutical composition comprising a molecule targeted to a tissue selected from the group consisting of pulmonary circulation, intestinal circulation, pulmonary *airways, lumen of the small intestine, dendritic cells in the skin and T cell areas of lymphoid organs, thymus, and brain, which molecule is conjugated to
20 a DEC-ligand. Preferably, the DEC-ligand is selected from the group consisting of a carbohydrate that binds DEC and an anti-DEC antibody, and a pharmaceutically acceptable carrier. In specific embodiments, the molecule is selected from the group consisting of an anti-cancer drug, an anti-viral drug, an antibiotic, an anti-parasitic drug, and an anti-inflammatory drug.

25

In another aspect of the invention related to targeting, a recombinant vector for introduction of a gene into cells selected from the group consisting of dendritic cells, thymic epithelial cells, lung epithelial cells, small intestine epithelial cells, and brain capillary cells comprising a DNA vector conjugated to a DEC-ligand is
30 provided, wherein the DEC-ligand is selected from the group consisting of a carbohydrate that binds DEC and an anti-DEC antibody. In specific embodiments,

the DNA vector is selected from the group consisting of a viral vector, a liposome vector, and a naked DNA vector.

In yet a further embodiment, grounded on the ability to target a molecule to
5 dendritic cells, the present invention provides a vaccine comprising an antigen
from a pathogen conjugated to a DEC-ligand, wherein the DEC-ligand is selected
from the group consisting of a carbohydrate that binds DEC and an anti-DEC
antibody, and an immune stimulator. Examples of pathogens include, but are not
10 limited to, a virus, a bacterium, a parasite, and a tumor. The immune stimulator
may be selected from the group consisting of a cytokine, a lymphokine, and an
adjuvant. In particular, the invention advantageously provides for targeting a
molecule that is either a poor immunogen, or that is not immunogenic at all, to
dendritic cells for efficient processing (as DEC is shown herein to be associated
15 with antigen processing mechanisms of dendritic cells) and presentation to
responsive T lymphocytes.

Alternatively, the invention provides a composition to induce immune suppression
comprising an autoantigen or an allergen conjugated to a DEC-ligand, wherein the
DEC ligand is selected from the group consisting of a carbohydrate that binds
20 DEC and an anti-DEC antibody, with the proviso that the composition lack
immune stimulatory agents. By targeting an autoantigen or allergen to dendritic
cells without including stimulatory agents, *e.g.*, cytokines, lymphokines, or
adjuvants, the quiescent dendritic cells can process and present antigen.
Presentation of antigen by quiescent dendritic cells is believed to induce antigen-
25 specific T cell anergy or immune tolerance. The autoantigen may be selected
from the group consisting of myelin basic protein, collagen or a fragment thereof,
DNA, a nuclear protein, a nucleolar protein, a mitochondrial protein, and a
pancreatic β -cell protein.

30 It is a primary object of the instant invention to provide ligands for DEC.

Accordingly, an important corollary object of the invention is to identify ligands that specifically bind DEC.

It is a further object to provide nucleic acids encoding DEC.

5

A related object is to express nucleic acids encoding DEC, or a portion thereof comprising a carbohydrate binding portion of a DEC lectin domain.

These and other objects of the present invention can be readily appreciated by
10 reference to the following Drawings, Detailed Description of the Invention, and Examples.

BRIEF DESCRIPTION OF THE DRAWINGS

15 **FIGURE 1. The apparent mass of the antigen bound by NLDC-145 is 205 kDa.** (A) Immunoprecipitation of (³⁵S)methionine-cysteine-labeled bone marrow DC extracts with immobilized NLDC-145 (*right*) reveals an actively synthesized antigen with an apparent mass > 200 kDa. This antigen is not precipitated by immobilized nonspecific rat IgG2a (*left*). (B) NLDC-145 binds an antigen of 205
20 kDa in non-reducing Western blots of crude thymic detergent extract. Three-fold serial dilutions of extract, starting at 0.17 thymic equivalents per lane, were loaded onto duplicate gels, in the absence (*left filter*) or presence (*right strip*) of 5% (v/v) 2-mercaptoethanol, and blotted to nitrocellulose. Filters were probed with 10 µg/ml of NLDC-145 IgG, then peroxidase-conjugated anti-rat IgG. Staining patterns were
25 visualized by ECL. No bands were observed on the reducing gel. STD: positions of prestained broad-range molecular mass markers (Bio-Rad) traced from the original filter onto the developed film.

FIGURE 2. Summary: purification of DEC-205 from thymi. All steps were
30 performed at 0-4°C. Leupeptin and PMSF were added to ice-cold buffers just before use.

FIGURE 3. Analysis of purified DEC-205 and step yields during the purification. (A) Reducing 8% acrylamide SDS-PAGE analysis of 5 μ g of purified protein, stained first with Coomassie Brilliant Blue R-250 (left), then counterstained with silver (right). (B) Isovolumic Western blot of key fractions from the purification, stained with 10 μ g/ml of NLDC-145 IgG. Fractions in lanes 1-4 and 6-8 were diluted to the volume of the post-nuclear supernatant, so that the intensities of their 205 kDa bands could be compared and yields estimated. Lane 10: intentional five-fold increase in antigen concentration, to demonstrate a "ladder" of minor mAb-reactive bands ranging down to about 80 kDa in apparent mass. Abbreviations: nuc, nuclear; memb, membrane; extr, 0.5% NP-40 extract; nonads, individual nonadsorbed fractions from early (nonads-5) and late (nonads-15) in the column loading process.

FIGURE 4. DEC-205 is an integral membrane protein with a pI of 7.5. (A) Immunoblot of thymic membrane proteins solubilized with detergent, 1 M KCl or 100 mM Na₂CO₃, pH 11.5 (lanes 1, 2, 3), and proteins initially insoluble in the high salt and high-pH buffers, but then released from membranes with detergent (lanes 4 and 5). The filter was stained with 10 μ g/ml of NLDC-145 IgG. (B) Isoelectric focusing of 10 μ g of purified DEC-205 under denaturing conditions. A single lane from a silver-stained slab gel is shown, with pH values assigned after elution of ampholytes from a neighboring unstained lane.

FIGURE 5. Studies of the carbohydrates bound to DEC-205. (A) DEC-205 is a glycoprotein. Purified 205 kDa protein, transferrin (Tf, positive control), and creatinase (cre, negative control) were electroblotted to nitrocellulose, and the filter was oxidized with NaIO₄, converting *vic*-diols within sugars to immobilized aldehydes. Reaction with a digoxigenin (DIG)-labeled hydrazide, followed by an anti-DIG antibody conjugated to alkaline phosphatase, revealed the positions of glycoproteins on the blot. Like transferrin, but unlike creatinase, DEC-205 stains for sugar. (B) The glycans on DEC-205 comprise about 7 kDa of its apparent molecular mass. Apotransferrin (aTf) and DEC-205 were either treated (+) or not

(-) with anhydrous trifluoromethanesulfonic acid (TFMSA), to nonselectively hydrolyze protein-bound carbohydrates. Both treated proteins exhibited increased electrophoretic mobility, corresponding to a 5 kDa loss of apparent mass by apotransferrin, and a 7 kDa loss by DEC-205. (C) FACE analysis of N-linked
 5 glycans released from DEC-205 with PNGase F. Eight bands are resolved, migrating between 5.1 and 10.1 glucose units. (Glc)₅, (Glc)₁₀: positions of selected bands in a standard oligo-glucose ladder. (D) Exoglycosidase digestions and FACE analysis of the mixture of N-linked glycans released from DEC-205. Lane 1: Undigested N-linked oligosaccharides (the dark band at (Glc)₅ in lanes 1-5 is a
 10 detergent artifact). Lane 2: digested with α -galactosidase. Lane 3: digested with α -galactosidase plus NANase III. Lane 4: digested with the previous 2 enzymes plus β -galactosidase. Lane 5: digested with the previous 3 enzymes plus β -N-acetylhexoseaminidase. Lane 6: as for lane 5, but 2-fold higher concentration of β -N-acetylhexoseaminidase. Lane 7: digested with the previous 4 enzymes plus α -
 15 mannosidase. Lane 8: as for lane 7, but 2-fold higher concentration of α -mannosidase plus α -fucosidase. Lane 9: mannosylchitobiose core standards: FC, fucosylated core; C, non-fucosylated core. (E) Summary of findings from lectin staining and FACE analysis. Two fucosylated core structures are present, with and without bisecting GlcNAc. Further heterogeneity at the termini produces the 8
 20 glycan variants observed in (C).

FIGURE 6. N-terminal amino acid sequence of DEC-205, and blotting by polyclonal antibodies. (A) The amino-terminal sequence (SEQ ID NO: 2), as determined by two different core facilities. A peptide spanning the first 19 residues
 25 was synthesized and coupled to KLH for use as an immunogen. (B) Preclearing study: NLDC-145 specifically depletes the 205 kDa bands detected by both polyclonal antibodies. Immunoblots of crude thymic membrane extract, a depleted fraction produced by passing the same extract over the NLDC-145 immunoaffinity column twice, and material eluted from the column. Filters were stained with: 10
 30 μ g/ml of NLDC-145 IgG, 0.1 μ g/ml of anti-N-terminal peptide IgG, and 0.1 μ g/ml of anti-DEC-205 IgG. All three antibodies bind the same protein.

FIGURE 7. Schematic representation of DEC-205.

FIGURE 8. Sequence of murine DEC-205 and related proteins. The predicted amino acid sequence of murine DEC-205 (SEQ ID NO:3) is aligned with the sequences of the bovine PLA2 receptor (SEQ ID NO:4) and the human macrophage mannose receptor (SEQ ID NO:5). Amino acid positions where there is identity among all three proteins are shaded. Protein domains are separated, and consensus amino acids that define C-type CRDs (Weis et al., Science 254:1608-15) are indicated below the relevant sequence as follows: invariant amino acids are shown in single letter code, θ = aliphatic, χ = aliphatic or aromatic, ϕ = aromatic, Z = E or Q, B = D or N, Ω = D, N, E or Q. The two missing cysteines in CRD 8 are highlighted with a *. Peptide sequences determined by automated Edman degradation from purified DEC-205 protein are overlined and numbered (N indicates amino terminal, T indicates peptides generated with Trypsin, and L indicates peptides generated with endoproteinase lys-C).

FIGURE 9. Comparison of carboxyl-terminal cytoplasmic domain sequences of human (*top*) (SEQ ID NO:1) and murine (*bottom*) (SEQ ID NO:6) DEC-205. Regions of identity are underlined; regions of similarity are italicized.

FIGURE 10. DEC-205 Expression. Expression of DEC-205 in mouse tissues and transfected Cos-7 cells. A. Northern blot of poly-A⁺ A extracted from the indicated tissues. Symbols: Br, brain mRNA; DC, dendritic cell mRNA; Ht, heart mRNA; Kd, kidney mRNA; Lv, liver mRNA; LN, lymph node mRNA; Sk, skin mRNA; Thm thymus mRNA; tg, tongue mRNA.

FIGURE 11. Endocytosis of DEC-205. Ultrastructural analysis of DEC-205 on dendritic cells with polyclonal rabbit anti-DEC-205 F(ab)₂ fragments and 10nm gold-labeled goat anti-rabbit IgG (Amersham). The bars represent 100 μ m. Symbols: MVV, multivesicular endosome; Ly, Lysosome; CP, coated pit; 0', fixation at time zero; 1', fixation after a one-minute incubation at 37°C; 5', fixation

after a five-minute incubation at 37°C; 20', fixation after a twenty-minute incubation at 37°C; 60', fixation after a sixty-minute incubation at 37°C.

FIGURE 12. Antigen Presentation. Antigen presentation by Dendritic cells incubated with rabbit anti-DEC-205 antibodies or non-reactive rabbit antibody controls. IL-2 production by the 2R.50 cells is plotted against the concentration of antibody in the cultures on a log scale. The error bars indicate the standard deviation from the mean. Symbols: anti-DEC-205, cultures that received the indicated amount of rabbit anti-DEC-205 polyclonal IgG; anti-IgG2a, cultures that received the indicated amount of IgG2a specific polyclonal rabbit antibodies; IgG, cultures that received the indicated amount of non-immune rabbit IgG.

FIGURE 13. Selective staining of Langerhans cells with monoclonal and polyclonal antibodies to DEC-205. Cultured epidermal cells were double-labeled with a PE-tagged mAb to class II MHC proteins (y axis) and multiple antibodies to leukocyte antigens, followed by FITC-anti-Ig (x axis). The mean FITC fluorescence intensity for the MHC-II (+) DCs (e.g., *arrows* in *E* and *H*) is shown in the upper right corner of each panel.

(*A-D*) *Specificity*: Langerhans cells stain for DEC-205, but not for macrophage, B cell or T cell antigens. Rat IgG2a hybridoma supernatants were applied.

(*E-X*) *Titration*s of the monoclonal and polyclonal reagents used in subsequent studies:

(*E-G*) Graded doses of NLDC-145 mAb (0.6, 2 and 6 µg/ml).

(*H*) Polyclonal, nonimmune rat IgG2a (6 µg/ml).

(*I-X*) Rabbit IgGs and F(ab')₂ fragments at 3, 10, 30 and 100 µg/ml

FIGURE 14. Trypsin sensitivity and resynthesis of DEC-205 epitopes.

Langerhans cells (*A-F*) or lymph node B cells (*G-L*) that had been cultured overnight were either exposed to 0.25% trypsin for 30 min on ice, or were not treated. The lymph node B cells had been stimulated with LPS to sustain viability. Cells were either stained immediately (*d1*) or after an additional day of culture

(d2). The antibodies were: 30 $\mu\text{g/ml}$ anti-DEC-205 or nonimmune F(ab')_2 fragments (A, C, E, G, I, and K); 2 $\mu\text{g/ml}$ NLDC-145 or nonimmune rat IgG2a; or anti-CD45 (clone M1/9, rat IgG2a) hybridoma supernatant (B, D, F, H, J, and L).

- 5 **FIGURE 15. Expression of DEC-205 by fresh and cultured dendritic cells (arrows) from spleen and skin.** Spleen DCs, enriched in the low-density fraction of spleen cells, were identified with anti-CD11c (y axis, A-H) and counterstained with: NLDC-145; F(ab')_2 fragments of the anti-DEC-205 polyclonal; and corresponding nonimmune controls. Staining was performed either immediately
10 after flotation (fresh), or after overnight culture. Fresh and cultured Langerhans cells, identified in an epidermal suspension with a mAb to class II MHC proteins (y axis, I-P), are shown for comparison.

- FIGURE 16. Expression of class II MHC proteins and DEC-205 by bone**
15 **marrow DCs grown from progenitors in the presence of GM-CSF.** On days 6, 7 and 8 of culture, cells were examined both by flow cytometry (A-F) and on cytocentrifuge slides (G-I) after staining with: 10 $\mu\text{g/ml}$ of TIB-120 (clone M5/114, anti I-A^{b,d,q}, anti I-E^{d,k}) or nonimmune IgG (A, C, E); 30 $\mu\text{g/ml}$ of anti-DEC-205 or nonimmune IgG (B, D, F); or 2 $\mu\text{g/ml}$ of NLDC-145 (G, H, I). (d6)
20 At day 6, the cultures contained large proliferating cell aggregates that expressed heterogeneous levels of class II MHC proteins and little DEC-205. The aggregates were dislodged from plastic-adherent stromal cells. (d7, d8) Over two subsequent days of culture, the aggregates released large numbers of nonadherent cells with typical dendritic morphology, abundant class II MHC proteins, and high levels of
25 DEC-205.

- FIGURE 17. Expression of DEC-205 on peritoneal cells.** Peritoneal cells, either resident or in exudates elicited with the indicated proinflammatory agents, were stained with 30 $\mu\text{g/ml}$ of anti-DEC-205 or nonimmune F(ab')_2 fragments and
30 FITC-anti-rabbit F(ab')_2 . The cells were then counterstained with PE-tagged mAbs to macrophages, B cells and T cells. Shown here is staining by PE-anti-Mac-

1/CD11b (mAb M1/70, y axis). The Mac-1^{bright} cells are macrophages (*arrowheads*), the Mac-1^{dim} cells are B cells (*arrows*) and the Mac-1 negative cells, T cells. Abbreviations: Con A, concanavalin A; TGC, thioglycollate; BCG, *Mycobacterium bovis* Bacille Calmette-Guérin.

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FIGURE 18. Expression of DEC-205 by leukocytes in fresh cell suspensions from three organs. B cells are arrowed. Cells from spleen (A-J), bone marrow (K-T), and peripheral blood (U-δ) were stained with PE-tagged antibodies to subsets of leukocytes (y axis), and counterstained with 30 µg/ml of nonimmune (A-E, K-O, and U-Y) or anti-DEC-205 (F-J, P-T, and Z-δ) F(ab')₂ fragments (x axis, FITC). The PE-labeled mAbs reacted with granulocytes (RB6-8C5, anti Gr-1), the integrin CD11b, abundant on granulocytes and macrophages (M1/70, anti-Mac-1), B cells (RA3-6B2, anti-B220/CD45RB), T cells (53-2.1, anti-Thy-1.2/CD90), and class II MHC proteins (AMS-32.1, anti- I-A^d).

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FIGURE 19. Immunoblot. Graded doses of whole-cell NP-40 extracts of bone marrow dendritic cells (BMDC), bulk splenic leukocytes (SPL, ca. 65% B cells) and resident peritoneal cells (PC, ca. 70% B cells, 30% macrophages) were transferred to a filter. The filter was stained with 10 µg/ml of NLDC-145 IgG.

20 BMDCs express roughly 10 times more DEC-205 per cell than splenic B cells, and roughly 50 times more than peritoneal B cells.

FIGURE 20. Inability of antibodies to DEC-205 to block dendritic cell stimulatory activity *in vitro*. A mixed leukocyte reaction, where graded doses of mitomycin C-treated spleen dendritic cells were added to 3 x 10⁵ allogeneic lymph node cells in the continuous presence of 10 µg/ml of each of the indicated antibodies, except for anti-DEC-205, which was used at 30 µg/ml. Only the mAb to the costimulator protein B7-2 (clone GL-1) inhibited T cell proliferation. Anti-Igβ: negative control polyclonal to a surface Ig-associated signalling protein on B

25

30 cells (Sanchez et al., 1993, J. Exp. Med. 178:1049).

FIGURE 21. Expression of DEC-205 in the thymus and in lymph nodes. (a-c): Low power of thymus cortex and medulla (M), stained with: monoclonal NLDC-145 (a); polyclonal anti-DEC-205 F(ab')₂ fragments (b); and polyclonal anti-DEC-205 IgG (c), all at 10 µg/ml, and counterstained with hematoxylin.

- 5 Presumptive dendritic cells (*arrowheads*) are scattered throughout the medulla, but the strongest thymic staining is on cortical epithelium. (d-f): Low power views of a mesenteric lymph node, showing a B cell follicle (B), the T cell area of the deep cortex (T), and the medulla (M), stained with: mAb NLDC-145 (d), polyclonal anti-DEC-205 F(ab')₂ fragments (e), and polyclonal anti-DEC-205 IgG (f).
- 10 Darkly-stained dendritic cells are distributed throughout the T cell areas. (g-i): Higher power views to show the distribution of DEC-205 at the junction of the thymic cortex and medulla (g), within the deep cortex of a lymph node (h, a venule is *arrowed*), and in B cell follicles (i, no hematoxylin counterstain).
- 15 **FIGURE 22. Expression of DEC-205 in the spleen.** (a-c): Low power views of a splenic white pulp nodule, stained with antibodies to: B cells (rabbit anti-Igβ, a); DEC-205 (polyclonal anti-DEC-205 IgG, b), and class II MHC proteins (mAb M5/114, c). The central arteries within the T cell areas are arrowed. The T cell areas contain few B cells (a, anti-Igβ), but numerous scattered DEC-205- and class
- 20 II MHC-positive dendritic cells (b-c). B cell follicles are denoted with a "B", and the marginal sinus by arrowheads. (d-e): Higher power views of splenic T cell areas (periarterial sheaths, central arteries are arrowed) stained with: mAb NLDC-145 (d), polyclonal anti-DEC-205 (e), and anti-class II MHC (f). Staining for DEC-205 has a punctate quality, in addition to the more prominent staining of
- 25 dendritic cell bodies.

FIGURE 23. Expression of DEC-205 in several nonlymphoid organs. (a-d): Brain capillaries (*arrows*, a-c) and small arteries (*arrow*, d), stained with: mAb NLDC-145 (a), polyclonal anti-DEC-205 F(ab')₂ fragments (b), and polyclonal anti-DEC-205 IgG (c-d). (e-h): Lung, showing anti-DEC-205 staining of airway epithelium (*arrows*, e and h), isolated cells within the lung parenchyma

(*arrowheads*, *g* and *h*), and some presumptive alveolar macrophages (*, *h*). Class II MHC proteins (*f*) are not evident within airway epithelium, but there are many positive profiles surrounding the airways (*arrowheads*, *f*). (*i*): An extruded plug of bone marrow. Lacy stromal cells (*arrows*) express low levels of DEC-205. The
 5 darker staining of round cells is background staining by peroxidase-expressing eosinophils. (*j*): Tongue, showing DEC-205 staining of a minority of presumptive Langerhans cells (*arrows*) at suprabasal levels within the oral epithelium, shown as an example of a stratified squamous epithelium. (*k,l*): Jejunum: DEC-205 is expressed by the absorptive epithelial cells of the intestinal villi, with the highest
 10 levels observed at the apices of the villi. Numerous cells within the lamina propria also stain darkly, but this staining is again a background of eosinophil peroxidase.

FIGURE 24. Tissue distribution of DEC-205 by immunoblotting. Lysates of the indicated organs were blotted to compare relative levels of expression of DEC-
 15 205 protein (*A*, filter stained with mAb NLDC-145) and the LAMP-1 lysosomal membrane antigen (*B*, filter stained with mAb 1D4B). Fifty μ g of total protein were loaded in each lane.

**FIGURE 25. Amino acid and nucleotide sequence comparisons for human
 20 and murine DEC-205.** (A) Matrix plot (pam 250 matrix) of translated murine (y-axis) and human (x-axis) DEC-205 amino acid sequences. The window size for this plot was 60, the minimum percent score was 60, and the hash value is 2. (B) Matrix plot (DNA identity matrix) of murine (y-axis) and human (x-axis) DNA sequences. The window size was 60, the minimum percent score was 65, the hash
 25 value was 6, and the jump value was 1. Both strands were evaluated.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to an integral membrane protein, termed herein
 30 "DEC," found primarily on dendritic cells, but also found on B cells, brain capillaries, bone marrow stroma, epithelia of intestinal villi and pulmonary airways,

as well as cortical epithelium of the thymus and the dendritic cells in the T cell areas of peripheral lymphoid organs. In addition, trace amounts of this protein are found in organs like the liver, heart, and kidney. The murine and human counterparts of DEC have an apparent molecular mass of 205 kDa, the murine counterpart has an isoelectric point at pH 7.5, and carbohydrates comprise about 7 kDa of the total mass of murine DEC. The carbohydrates appear to consist of eight distinct but related biantennary N-linked glycans, with no O-linked glycans present. Because the protein has been found predominantly on Dendritic cells and thymic Epithelial Cells, and has a molecular weight of 205 kDa, it is termed DEC-205. Although characterization of the murine and human counterparts of DEC demonstrates the presence of ten carbohydrate binding domains, with a high degree of homology, it is possible that DEC from other species may have more or fewer such domains. Similarly, DEC may be expressed by other cell types, such as epithelial cells from other tissues or organs.

The invention further relates to cloning of the gene encoding DEC-205, and characterization of the encoded protein. The sequence information indicates that DEC-205 is a receptor with ten C-type lectin domains, which is homologous, or similar, to the macrophage mannose receptor and other related receptors that bind carbohydrates and mediate endocytosis. The human counterpart also appears to have lectin domains. Accordingly, DEC is believed to have a corresponding number of lectin domains, and to be involved in antigen processing by dendritic cells.

Still another aspect of the present invention is the identification of a human DEC protein and gene encoding it.

Another characteristic of DEC, based on the deduced amino acid sequence information obtained from cloning the *dec* cDNA is a unique coated pit localization consensus domain or motif on the cytoplasmic tail of the protein, which, as shown

in Figure 9, has regions of homology and regions of dissimilarity between the two counterpart proteins.

It has been further discovered that DEC-205 is rapidly internalized via coated
5 vesicles, and delivers bound substances to a multivesicular endosomal compartment that resembles the MHC class-II containing vesicles implicated in antigen processing. The invention is also based, in part, on the further discovery that rabbit antibody specific for DEC-205 was efficiently processed by dendritic cells and presented to rabbit-specific T cell clones.

10

Accordingly, and most importantly, the present invention is directed to identification of additional ligands of the DEC-205 receptor, which can be advantageously targeted to dendritic cells and other cells that bear DEC-205. Targeting antigens for presentation by dendritic cells can provide for tolerance
15 when the dendritic cells are quiescent, or for immune stimulation (*i.e.*, vaccination) when the dendritic cells are activated, *e.g.*, by stimulation with a cytokine or lymphokine, such as colony stimulating factor (CSF).

Furthermore, the presence of DEC on epithelial cells suggests an important role in
20 trans-epithelial transport of molecules, *e.g.*, from the basolateral surface of the lung respiratory epithelium into lung airways or the basolateral surface of the intestinal epithelium into the lumen of the intestines, and from the apical (luminal) surface of these epithelial to the pulmonary or intestinal circulation, respectively. Thus, the invention provides for parenteral delivery of pharmacological agents, *e.g.*,
25 antibiotics, to infections of the lung or the intestines, by targeting the pharmacological agent with a ligand to DEC, and for systemic delivery of pharmacological agents by aerosolization and inhalation via the lungs, or ingestion and absorption via the intestines.

30 In a further aspect, the invention provides for targeting pharmacological agents to cross the blood brain barrier via DEC located on the brain capillaries.

Accordingly, various terms are used throughout this specification, which have the meanings as defined below.

- The term "candidate ligand" is used herein to refer to a molecule under
- 5 consideration of test for its ability to specifically bind to DEC. As discussed in greater detail, *infra*, candidate ligands include, but are by no means limited to, saccharides (*i.e.*, sugars, carbohydrates, or glycans). The term "ligand" as used herein can also refer to an antibody reactive with DEC.
- 10 A molecule is "antigenic" when it is capable of specifically interacting with an antigen recognition molecule of the immune system, such as an immunoglobulin (antibody) or T cell antigen receptor. An antigenic polypeptide contains at least about 5, and preferably at least about 10, amino acids. An antigenic portion of a
- 15 molecule can be that portion that is immunodominant for antibody or T cell receptor recognition, or it can be a portion used to generate an antibody to the molecule by conjugating the antigenic portion to a carrier molecule for immunization. A molecule that is antigenic need not be itself immunogenic, *i.e.*, capable of eliciting an immune response without a carrier.
- 20 A composition comprising "A" (where "A" is a single protein, DNA molecule, vector, recombinant host cell, etc.) is substantially free of "B" (where "B" comprises one or more contaminating proteins, DNA molecules, vectors, etc.) when at least about 75% by weight of the proteins, DNA, vectors (depending on the category of species to which A and B belong) in the composition is "A".
- 25 Preferably, "A" comprises at least about 90% by weight of the A+B species in the composition, most preferably at least about 99% by weight. It is also preferred that a composition, which is substantially free of contamination, contain only a single molecular weight species having the activity or characteristic of the species of interest.

The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. In a specific embodiment, as used herein, the term

5 "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids,

10 such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water or aqueous solution saline solutions and aqueous dextrose and glycerol solutions are preferably employed as carriers, particularly for injectable solutions. Suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by

15 E.W. Martin.

The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to reduce by at least about 15 percent, preferably by at least 50 percent, more preferably by at least 90 percent, and most preferably prevent, a clinically

20 significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in the host.

The term "adjuvant" refers to a compound or mixture that enhances the immune

25 response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or

30 cellular immune response. Adjuvants include, but are not limited to, complete Freund's adjuvant, incomplete Freund's adjuvant, saponin, mineral gels such as

aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (*bacille Calmette-Guerin*) and *Corynebacterium parvum*. Preferably, the adjuvant
5 is pharmaceutically acceptable.

Various abbreviations used throughout this specification include: PBS, phosphate-buffered saline; mAb, monoclonal antibody; SPF, specific pathogen-free; PMSF, phenylmethylsulfonyl fluoride; DIFP, diisopropyl fluorophosphate; FACE,
10 fluorophore-assisted carbohydrate electrophoresis.

Genes Encoding DEC, or Fragments, Derivatives, Chimeras, or Analogs Thereof

The present invention contemplates isolation of a gene encoding a functional portion of a DEC receptor of the invention, including a full length, or naturally
15 occurring form of DEC, and any antigenic fragments thereof from any animal, particularly mammalian or avian, and more particularly human, source. As used herein, the term "gene" refers to an assembly of nucleotides that encode a polypeptide, and includes cDNA and genomic DNA nucleic acids. In specific
20 DNA is provided (SEQ ID NO:8); also provided are deduced coding sequences for both murine and human DEC polypeptides having the amino acid sequences depicted in SEQ ID NO:3 and SEQ ID NO:8, respectively, are provided.

Accordingly, there may be employed conventional molecular biology,
25 microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (herein
"Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II
30 (D.N. Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed. 1984); *Nucleic Acid Hybridization* [B.D. Hames & S.J. Higgins eds. (1985)]; *Transcription And*

Translation [B.D. Hames & S.J. Higgins, eds. (1984)]; *Animal Cell Culture* [R.I. Freshney, ed. (1986)]; *Immobilized Cells And Enzymes* [IRL Press, (1986)]; B. Perbal, *A Practical Guide To Molecular Cloning* (1984); F.M. Ausubel et al. (eds.), *Current Protocols in Molecular Biology*. John Wiley & Sons, Inc. (1994).

5

Therefore, if appearing herein, the following terms shall have the definitions set out below.

- A "nucleic acid molecule" refers to the phosphate ester polymeric form of
10 ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA
15 molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (e.g., restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein
20 according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation.
- 25 A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (*see* Sambrook et al., *supra*). The conditions of temperature and ionic strength determine the "stringency" of the
30 hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m of 55°, can be used. *e.g.*,

5x SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5x SSC, 0.5% SDS). Moderate stringency hybridization conditions correspond to a higher T_m , e.g., 40% formamide, with 5x or 6x SCC. High stringency hybridization conditions correspond to the highest T_m , e.g., 50% formamide, 5x or 6x SCC.

- 5 Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology
- 10 between two nucleotide sequences, the greater the value of T_m for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (*see* Sambrook et al.,
- 15 *supra*, 9.50-0.51). For hybridization with shorter nucleic acids, *i.e.*, oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (*see* Sambrook et al., *supra*, 11.7-11.8). Preferably a minimum length for a hybridizable nucleic acid is at least about 10 nucleotides; more preferably at least about 15 nucleotides; most preferably
- 20 the length is at least about 20 nucleotides.

- A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide in a cell *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The boundaries of the coding
- 25 sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. If the coding sequence is intended for expression
- 30 in a eukaryotic cell, a polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

Expression control sequences, e.g., transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, terminators, and the like, that provide for the expression of a coding sequence in a host cell. In eukaryotic cells, polyadenylation signals are control sequences.

5

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and
10 extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined for example, by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA
15 polymerase.

A coding sequence is "under the control of" or "operatively associated with" a transcriptional and translational control sequence in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and
20 translated into the protein encoded by the coding sequence.

A "signal sequence" is included at the beginning of the coding sequence of a protein to be expressed on the surface of a cell. This sequence encodes a signal peptide, N-terminal to the mature polypeptide, that directs the host cell to
25 translocate the polypeptide. The term "translocation signal sequence" is used herein to refer to this sort of signal sequence. Translocation signal sequences can be found associated with a variety of proteins native to eukaryotes and prokaryotes, and are often functional in both types of organisms.

30 As used herein, the term "sequence homology" in all its grammatical forms refers to the relationship between proteins that possess a "common evolutionary origin,"

including proteins from superfamilies (*e.g.*, the immunoglobulin superfamily) and homologous proteins from different species (*e.g.*, myosin light chain, etc.) (Reeck et al., 1987, Cell 50:667).

- 5 Accordingly, the term "sequence similarity" in all its grammatical forms refers to the degree of identity or correspondence between nucleic acid or amino acid sequences of proteins that do not share a common evolutionary origin (*see* Reeck et al., *supra*).
- 10 Two DNA sequences are "substantially homologous" or "substantially similar" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks,
- 15 or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, *e.g.*, Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.
- 20 Similarly, two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 70% of the amino acids are identical, or functionally identical. Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, *Version 7*, Madison, Wisconsin) pileup
- 25 program.

The term "corresponding to" is used herein to refer similar or homologous sequences, whether the exact position is identical or different from the molecule to which the similarity or homology is measured. Thus, the term "corresponding to"

30 refers to the sequence similarity, and not the numbering of the amino acid residues or nucleotide bases.

A gene encoding DEC, whether genomic DNA or cDNA, can be isolated from any source, particularly from a human cDNA or genomic library. Methods for obtaining DEC gene are well known in the art, as described above (*see, e.g.,* Sambrook et al., 1989, *supra*). In specific embodiment, *infra*, a cDNA encoding
5 murine DEC-205 is isolated from a dendritic cell library. In addition, probes derived from the murine gene were used to isolate the corresponding human *dec* cDNA and the murine genomic *dec* gene.

Accordingly, any animal cell potentially can serve as the nucleic acid source for the
10 molecular cloning of a *dec* gene. The DNA may be obtained by standard procedures known in the art from cloned DNA (*e.g.,* a DNA "library"), and preferably is obtained from a cDNA library prepared from tissues with high level expression of the protein (*e.g.,* a dendritic cell cDNA or thymic epithelial cDNA library, since these are the cells that evidence highest levels of expression of DEC),
15 by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from the desired cell (See, for example, Sambrook et al., 1989, *supra*; Glover, D.M. (ed.), 1985, DNA Cloning: A Practical Approach, MRL Press, Ltd., Oxford, U.K. Vol. I, II). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions;
20 clones derived from cDNA will not contain intron sequences. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene.

In the molecular cloning of the gene from genomic DNA, DNA fragments are
25 generated, some of which will encode the desired gene. The DNA may be cleaved at specific sites using various restriction enzymes. Alternatively, one may use DNase in the presence of manganese to fragment the DNA, or the DNA can be physically sheared, as for example, by sonication. The linear DNA fragments can then be separated according to size by standard techniques, including but not
30 limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired *dec* gene may be accomplished in a number of ways. For example, if an amount of a portion of a *dec* gene or its specific RNA, or a fragment thereof, is available and can be purified and labeled, the generated

5 DNA fragments may be screened by nucleic acid hybridization to the labeled probe (Benton and Davis, 1977, Science 196:180; Grunstein and Hogness, 1975, Proc. Natl. Acad. Sci. U.S.A. 72:3961). For example, a set of oligonucleotides corresponding to the partial amino acid sequence information obtained for the DEC protein can be prepared and used as probes for DNA encoding DEC, as was done

10 in a specific example, *infra*, or as primers for cDNA or mRNA (*e.g.*, in combination with a poly-T primer for RT-PCR). Preferably, a fragment is selected that is highly unique to DEC of the invention. Those DNA fragments with substantial homology to the probe will hybridize. As noted above, the greater the degree of homology, the more stringent hybridization conditions can be used. In a

15 specific embodiment, the human *dec* cDNA was cloned using a 300 base-pair probe derived from the 3' coding sequence of murine *dec* cDNA. The human cDNA was obtained from a B lymphoma library, using high stringency hybridization conditions (0.1 SSC, 65°C). Thus, high stringency hybridization conditions are favored to identify a homologous *dec* gene from other species.

20

Further selection can be carried out on the basis of the properties of the gene, *e.g.*, if the gene encodes a protein product having the isoelectric, electrophoretic, amino acid composition, or partial amino acid sequence of DEC protein as disclosed herein. Thus, the presence of the gene may be detected by assays based on the

25 physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or DNA clones which hybrid-select the proper mRNAs, can be selected which produce a protein that, *e.g.*, has similar or identical electrophoretic migration, isoelectric focusing or non-equilibrium pH gel electrophoresis behavior, proteolytic digestion maps, or antigenic properties as

30 known for DEC. For example, the rabbit polyclonal antibody to murine DEC, described in detail *infra*, can be used to confirm expression of DEC, both murine

and human counterparts. In another aspect, a protein that has an apparent molecular weight of 205 kDa, and which is specifically digested to form a defined ladder (rather than a smear) of lower molecular weight bands, is a good candidate for DEC.

5

A *dec* gene of the invention can also be identified by mRNA selection, *i.e.*, by nucleic acid hybridization followed by *in vitro* translation. In this procedure, nucleotide fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified *dec* DNA, or may be
10 synthetic oligonucleotides designed from the partial amino acid sequence information. Immunoprecipitation analysis or functional assays (*e.g.*, tyrosine phosphatase activity) of the *in vitro* translation products of the products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments, that contain the desired sequences. In addition, specific mRNAs may be
15 selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against DEC, such as the rabbit polyclonal anti-murine DEC antibody described herein.

A radiolabeled *dec* cDNA can be synthesized using the selected mRNA (from the
20 adsorbed polysomes) as a template. The radiolabeled mRNA or cDNA may then be used as a probe to identify homologous *dec* DNA fragments from among other genomic DNA fragments.

The present invention also relates to cloning vectors containing genes encoding
25 analogs and derivatives of DEC of the invention, that have the same or homologous functional activity as DEC, and homologs thereof from other species. The production and use of derivatives and analogs related to DEC are within the scope of the present invention. In a specific embodiment, the derivative or analog is functionally active, *i.e.*, capable of exhibiting one or more functional activities
30 associated with a full-length, wild-type DEC.

In another aspect, a DEC protein of the invention can be prepared by substituting a lectin domain or domains from another protein, such as the mannose receptor of macrophages or the phospholipase receptor on muscle, for those found in DEC 205.

- 5 DEC derivatives can be made by altering encoding nucleic acid sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Preferably, derivatives are made that have enhanced or increased functional activity relative to native DEC. Alternatively, such derivatives may encode soluble fragments of DEC extracellular domain that have the same or
10 greater affinity for the natural ligand of DEC of the invention. Such soluble derivatives may be potent inhibitors of ligand binding to DEC.

Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a *dec* gene may be used in
15 the practice of the present invention. These include but are not limited to allelic genes, homologous genes from other species, and nucleotide sequences comprising all or portions of *dec* genes which are altered by the substitution of different codons that encode the same amino acid residue within the sequence, thus producing a silent change. Likewise, the DEC derivatives of the invention include,
20 but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence of a DEC protein including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence resulting in a conservative amino acid substitution. For example, one or more amino acid residues within the sequence can be substituted
25 by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar
30 neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include

arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Such alterations will not be expected to affect apparent molecular weight as determined by polyacrylamide gel electrophoresis, or isoelectric point.

5

The genes encoding DEC derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned DEC gene sequence can be modified by any of numerous strategies known in the art
10 (Sambrook et al., 1989, *supra*). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of DEC, care should be taken to ensure that the modified gene remains within the same translational reading frame as the DEC gene, uninterrupted
15 by translational stop signals, in the gene region where the desired activity is encoded.

Additionally, the DEC-encoding nucleic acid sequence can be mutated *in vitro* or *in vivo*, to create and/or destroy translation, initiation, and/or termination sequences, or
20 to create variations in coding regions and/or form new restriction endonuclease sites or destroy preexisting ones, to facilitate further *in vitro* modification. Preferably, such mutations enhance the functional activity of the mutated DEC gene product. Alternatively, deletion mutants can be produced that encode fragments of DEC, *e.g.*, one or a few of the lectin domains (*see* Taylor et al., 1992, J. Biol. Chem.
25 267:1719). Any technique for mutagenesis known in the art can be used, including but not limited to, *in vitro* site-directed mutagenesis (Hutchinson, C., et al., 1978, J. Biol. Chem. 253:6551; Zoller and Smith, 1984, DNA 3:479-488; Oliphant et al., 1986, Gene 44:177; Hutchinson et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:710), use of TAB® linkers (Pharmacia), etc. PCR techniques are preferred for site
30 directed mutagenesis (*see* Higuchi, 1989, "Using PCR to Engineer DNA", in *PCR*

Technology: Principles and Applications for DNA Amplification, H. Erlich, ed., Stockton Press, Chapter 6, pp. 61-70).

The identified and isolated gene can then be inserted into an appropriate cloning
5 vector. A large number of vector-host systems known in the art may be used. Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Examples of vectors include, but are not limited to, *E. coli*, bacteriophages such as lambda derivatives, or plasmids such as pBR322 derivatives or pUC plasmid derivatives, *e.g.*, pGEX
10 vectors, pmal-c, pFLAG, etc. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired
15 may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers may comprise specific chemically synthesized oligonucleotides encoding restriction endonuclease recognition sequences. Recombinant molecules can be introduced into host cells via transformation, transfection, infection, electroporation, etc., so that many copies of the gene sequence are generated.
20 Preferably, the cloned gene is contained on a shuttle vector plasmid, which provides for expansion in a cloning cell, *e.g.*, *E. coli*, and facile purification for subsequent insertion into an appropriate expression cell line, if such is desired. For example, a shuttle vector, which is a vector that can replicate in more than one type of organism, can be prepared for replication in both *E. coli* and *Saccharomyces*
25 *cerevisiae* by linking sequences from an *E. coli* plasmid with sequences from the yeast 2 μ plasmid.

In an alternative method, the desired gene may be identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for
30 the desired gene, for example, by size fractionation, can be done before insertion into the cloning vector.

Expression of DEC Polypeptides

The nucleotide sequence coding for DEC, or antigenic fragment, derivative or analog thereof, or a functionally active derivative, including a chimeric protein, thereof, can be inserted into an appropriate expression vector, *i.e.*, a vector which
5 contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. Such elements are termed herein a "promoter." Thus, the nucleic acid encoding DEC of the invention is operationally associated with a promoter in an expression vector of the invention. Both cDNA and genomic sequences can be cloned and expressed under control of such regulatory sequences.
10 An expression vector also preferably includes a replication origin.

The necessary transcriptional and translational signals can be provided on a recombinant expression vector, or they may be supplied by the native gene encoding DEC and/or its flanking regions.

15

Potential host-vector systems include but are not limited to mammalian cell systems infected with virus (*e.g.*, vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (*e.g.*, baculovirus); microorganisms such as yeast containing yeast vectors; or bacteria transformed with bacteriophage, DNA, plasmid DNA, or
20 cosmid DNA. The expression elements of vectors vary in their strengths and specificities. Depending on the host-vector system utilized, any one of a number of suitable transcription and translation elements may be used.

A recombinant DEC protein of the invention, or functional fragment, derivative,
25 chimeric construct, or analog thereof, may be expressed chromosomally, after integration of the coding sequence by recombination. In this regard, any of a number of amplification systems may be used to achieve high levels of stable gene expression (*See* Sambrook et al., 1989, *supra*).

The cell into which the recombinant vector comprising the nucleic acid encoding DEC is cultured in an appropriate cell culture medium under conditions that provide for expression of DEC by the cell.

- 5 Any of the methods previously described for the insertion of DNA fragments into a cloning vector may be used to construct expression vectors containing a gene consisting of appropriate transcriptional/translational control signals and the protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombination (genetic recombination).
- 10
- Expression of DEC protein may be controlled by any promoter/enhancer element known in the art, but these regulatory elements must be functional in the host selected for expression. Promoters which may be used to control DEC gene expression include, but are not limited to, the SV40 early promoter region (Benoist and Chambon, 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the
- 15 β -lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the *tac* promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25); see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADC (alcohol dehydrogenase) promoter, PGK
- 20 (phosphoglycerol kinase) promoter, alkaline phosphatase promoter; and the animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals.
- 25

- Expression vectors containing a nucleic acid encoding a DEC of the invention can
- 30 be identified by four general approaches: (a) PCR amplification of the desired plasmid DNA or specific mRNA, (b) nucleic acid hybridization. (c) presence or absence of selection marker gene functions, and (d) expression of inserted

sequences. In the first approach, the nucleic acids can be amplified by PCR to provide for detection of the amplified product. In the second approach, the presence of a foreign gene inserted in an expression vector can be detected by nucleic acid hybridization using probes comprising sequences that are homologous to an inserted marker gene. In the third approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "selection marker" gene functions (*e.g.*, β -galactosidase activity, thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign genes in the vector. In another example, if the nucleic acid encoding DEC is inserted within the "selection marker" gene sequence of the vector, recombinants containing the DEC insert can be identified by the absence of the DEC gene function. In the fourth approach, recombinant expression vectors can be identified by assaying for the activity, biochemical, or immunological characteristics of the gene product expressed by the recombinant, provided that the expressed protein assumes a functionally active conformation.

Once a particular recombinant DNA molecule is identified and isolated, several methods known in the art may be used to propagate it. Once a suitable host system and growth conditions are established, recombinant expression vectors can be propagated and prepared in quantity. As previously explained, the expression vectors which can be used include, but are not limited to, the following vectors or their derivatives: human or animal viruses such as vaccinia virus or adenovirus; insect viruses such as baculovirus; yeast vectors; bacteriophage vectors (*e.g.*, lambda), and plasmid and cosmid DNA vectors, to name but a few.

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage [*e.g.*, of signal sequence]) of proteins. Appropriate cell

lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce a nonglycosylated core protein product. However, the transmembrane DEC protein expressed in bacteria may not be properly folded.

- 5 Expression in yeast can produce a glycosylated product, although the pattern of glycosylation will likely differ from that obtained by expression in a mammalian cell. Expression in eukaryotic cells can increase the likelihood of "native" glycosylation and folding of a heterologous protein. Moreover, expression in mammalian cells, such as Chinese hamster ovary (CHO), African Green Monkey
- 10 COS cells, and fibroblast NIH-3T3 cells (*e.g.*, 293 cells), can provide a tool for reconstituting, or constituting, DEC activity. Furthermore, different vector/host expression systems may affect processing reactions, such as proteolytic cleavages, to a different extent.
- 15 In a preferred aspect of the invention, DEC is introduced into model epithelial cells, such as Madin-Darby canine kidney (MDCK) cells, for investigation of the efficacy and rate of trans-epithelial migration of ligands or molecules targeted to DEC. Alternatively, as set forth below, the *dec* gene can be introduced into epithelial or dendritic cells for gene therapy, either by *in vivo* or *ex vivo* gene
- 20 transfer.

Vectors are introduced into the desired host cells by methods known in the art, *e.g.*, transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, lipofection (lysosome fusion). use of a

25 gene gun, or a DNA vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

A recombinant DEC protein expressed as an integral membrane protein can be

30 isolated and purified by standard methods. Generally, the integral membrane protein can be obtained by lysing the membrane with detergents, such as but not

limited to, sodium dodecyl sulfate (SDS), Triton X-100, Nonidet P-40 (NP-40), digoxin, sodium deoxycholate, and the like, including mixtures thereof.

Solubilization can be enhanced by sonication of the suspension. In a specific embodiment, *infra*, DEC-205 is solubilized from thymic membrane pellets in a buffer containing 0.5% NP-40. Soluble forms of the protein can be obtained by
5 collecting culture fluid, or solubilizing inclusion bodies, *e.g.*, by treatment with detergent, and if desired sonication or other mechanical processes, as described above. The solubilized or soluble protein can be isolated using various techniques, such as polyacrylamide gel electrophoresis (PAGE), isoelectric focusing, 2-
10 dimensional gel electrophoresis, chromatography (*e.g.*, ion exchange, affinity, immunoaffinity, and sizing column chromatography), centrifugation, differential solubility, immunoprecipitation, or by any other standard technique for the purification of proteins. In a specific embodiment, *infra*, DEC-205 was purified using an affinity column with monoclonal antibody NLDC-145. In a another
15 embodiment, *infra*, DEC-205 was purified by immunoprecipitation with either monoclonal antibody NLDC-145.

Characterization of DEC Structure

Once a recombinant which expresses the DEC gene sequence is identified, and
20 expression of an abundance of the protein is achieved, the recombinant DEC product can be analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis, immunoassay, etc.

25 For example, the ability of the expressed protein, or a fragment comprising the cytoplasmic domain thereof, to mediate endocytosis and targeting to coated pits, and thence to endocytic vesicles associated with Class II MHC processing, can be determined. In one embodiment, *infra*, endocytosis was evaluated by electron microscopy, using an anti-DEC antibody and a gold-labeled secondary antibody
30 reactive with the anti-DEC antibody. In another embodiment, the ability to process

and present antigen is evaluated by assaying antibody-specific T cell proliferation in response to processing of anti-DEC antibody and a control non-specific antibody.

The structure of DEC of the invention can be analyzed by various methods known in the art. Preferably, the structure of the various domains, particularly the lectin binding and cytoplasmic domains, is analyzed. Structural analysis can be performed by identifying sequence similarity with other known proteins. The degree of similarity (or homology) can provide a basis for predicting structure and function of DEC, or a domain thereof. In a specific embodiment, sequence comparisons can be performed with sequences found in GenBank, using, for example, the FASTA and FASTP programs (Pearson and Lipman, 1988, Proc. Natl. Acad. Sci. USA 85:2444-48).

The protein sequence can be further characterized by a hydrophilicity analysis (*e.g.*, Hopp and Woods, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:3824). A hydrophilicity profile can be used to identify the hydrophobic and hydrophilic regions of the DEC protein.

Secondary structural analysis (*e.g.*, Chou and Fasman, 1974, Biochemistry 13:222) can also be done, to identify regions of DEC that assume specific secondary structures.

Manipulation, translation, and secondary structure prediction, as well as open reading frame prediction and plotting, can also be accomplished using computer software programs available in the art.

By providing an abundant source of recombinant DEC, the present invention enables quantitative structural determination of DEC, or domains thereof. In particular, enough material is provided for nuclear magnetic resonance (NMR), infrared (IR), Raman, and ultraviolet (UV), especially circular dichroism (CD), spectroscopic analysis. In particular NMR provides very powerful structural

analysis of molecules in solution, which more closely approximates their native environment (Marion et al., 1983, Biochem. Biophys. Res. Comm. 113:967-974; Bar et al., 1985, J. Magn. Reson. 65:355-360; Kimura et al., 1980, Proc. Natl. Acad. Sci. U.S.A. 77:1681-1685). Other methods of structural analysis can also be
5 employed. These include but are not limited to X-ray crystallography (Engstrom, A., 1974, Biochem. Exp. Biol. 11:7-13).

More preferably, co-crystals of DEC and a DEC-specific ligand can be studied. Analysis of co-crystals provides detailed information about binding, which in turn
10 allows for rational design of ligand agonists and antagonists. Computer modeling can also be used, especially in connection with NMR or X-ray methods (Fletterick, R. and Zoller, M. (eds.), 1986, Computer Graphics and Molecular Modeling, in *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York).

15

In yet a further embodiment, a putative DEC of the invention can be tested to determine whether it cross-reacts with an antibody specific for murine DEC-205. For example, the putative DEC can be reacted with a rabbit polyclonal antibody, as described in the Example, *infra*, to determine whether it binds. Alternatively, a
20 DEC protein can be used to generate antibodies, which can be tested for cross reactivity with DEC-205 from mice or human sources. The degree of cross reactivity provides information about structural homology or similarity of proteins.

The carbohydrate composition of DEC can be studied by various means known in
25 the art, including but not limited to, lectin binding, chemical analysis, immunoassay, immunochemical analysis (*e.g.*, by converting glycoconjugates to digoxigenin-labeled hydrazones after periodate oxidation of *vic*-diols), chemical deglycosylation, enzymatic deglycosylation, and exoglycosidase digestions followed by FACE (fluorophore-assisted carbohydrate electrophoresis) analysis.

30

Ligands for DEC

Most importantly, the present invention advantageously provides for identifying ligands of DEC, *e.g.*, carbohydrate ligands that bind to one or more of the lectin domains of DEC. Such ligands are especially useful for targeting binding to DEC.

5 As used herein, "ligand" has its ordinary meaning, *i.e.*, a molecule capable of specifically binding to a receptor, in this case DEC. As used herein, the term "carbohydrate ligand" refers to a carbohydrate or sugar that is capable of specifically binding DEC. Generally, such carbohydrates, alternatively termed herein "glycans," "saccharides," or "oligosaccharides," are the carbohydrate portion
10 of a glycoprotein.

Identification and isolation of a gene encoding DEC of the invention provides for expression of the receptor, or truncated portions thereof, in quantities greater than can be isolated from natural sources, in recombinant cells for classical receptor
15 binding experiments, or in indicator cells that are specially engineered to indicate the activity of a receptor expressed after transfection or transformation of the cells. According, the present invention contemplates identifying specific ligands DEC using various screening assays known in the art. The recombinantly expressed protein can comprise one or more DEC lectin domains, and may be a truncated
20 form of the native protein or portions of the native protein expressed as a chimeric construct with another protein.

Any screening technique known in the art can be used to screen for DEC ligands. The present invention contemplates screens for small molecule ligands or ligand
25 analogs and mimics, as well as screens for natural ligands that bind to DEC *in vivo*. In particular, the present invention provides for identification of carbohydrate groups that bind DEC, and more specifically, identification of carbohydrate groups that bind DEC with high affinity and specificity.

30 In a preferred aspect of the invention, detection of DEC ligands is accomplished by binding solubilized DEC or DEC fragments to columns prepared from sugars or

glycans conjugated to a solid phase support, such as SEPHAROSE (Taylor et al., 1992, J. Biol. Chem. 267:1719). In particular, the invention contemplates dissecting the ligand specificity of various of the lectin domains by expressing truncated mutant DEC proteins comprising only one or a few of the domains.

- 5 Alternatively, candidate glycans can be conjugated to a carrier protein, such as bovine serum albumin, which is labelled, *e.g.*, with ^{125}I , and binding detected to DEC or DEC fragments expressed by a cell, such as a recombinant cell as described *supra* (Taylor et al., *supra*). In yet another embodiment, binding of labeled glycan-carrier protein is evaluated in microtiter assays, as described (Taylor
10 and Drickamer, 1993, J. Biol. Chem. 268:399). Candidate carbohydrate ligands include, but are not limited to, mannose, fucose, N-acetyl-glucosamine, glucose, galactose, N-acetyl-galactosamine, to mention but a few such carbohydrates. Other ligand candidates include disaccharides, and larger order polysaccharides, *e.g.*, such as are recognized by various lectins.

15

- As used herein, the term "detection of binding" refers to any of the myriad techniques commonly employed to detect the association of one molecule with another, *i.e.*, DEC-ligand with DEC. These techniques include the immunoassay techniques discussed *infra*, or modifications thereof, and generally depend on
20 detecting association of a label conjugated with one of the binding entities, either the DEC-lectin containing polypeptide or the candidate ligand, with the other entity, which may be found on a solid phase support or a cell. However, detection of binding can be accomplished indirectly, by detecting the absence of a labeled binding entity, *e.g.*, from supernatant. In a further aspect, binding can be detected
25 by first removing unbound substances, followed by removing the labeled entity (*e.g.*, using a chaotropic agent) from the bound pair. These and other techniques for detecting binding of one entity to another are well known in the art.

- For solid phase or heterogeneous phase assays, one entity of the binding pair will
30 be irreversibly associated with a solid phase support, such as a bead (*e.g.*, SEPHAROSE), latex particle, chromatographic support, magnetic particle, silica

particle, silicon wafer, or a plastic microtiter plate. The term "irreversibly associated" refers to covalent or non-covalent binding, characterized by no dissociation, or a rate of dissociation that is so low in comparison to the assay time that it is virtually undetectable.

5

Knowledge of the primary sequence of DEC, and the similarity of that sequence with proteins of known function, can provide an initial clue as the inhibitors or antagonists of the protein. In the present instance, correlation of the deduced sequence of DEC with the sequences of mannose receptor of macrophage and phospholipase receptor on muscle, assisted characterization of DEC as a receptor with multiple lectin domains. Identification and screening of antagonists is further facilitated by determining structural features of the protein, *e.g.*, using X-ray crystallography, neutron diffraction, nuclear magnetic resonance spectrometry, and other techniques for structure determination, as described above.

15

In a specific embodiment, identification of carbohydrate ligands for DEC will be accomplished by attaching known glycans to a protein such as the classic neo-glycoprotein, bovine serum albumin, or ovalbumin, or creatinase. In particular, it is advantageous to use a protein that is not naturally glycosylated, so that only the effects of an added glycan are being assayed. The binding assay may comprise a classical binding assay, as described above, or may involve an antigen processing assay, by evaluating stimulation of antigen-specific T lymphocytes. In this regard, a large number of T cell lines and clones specific for BSA and ovalbumin are available: the ability of neo-glycosylated BSA or ovalbumin to efficiently stimulate specific T cell proliferation is indicative of the ability of the glycan conjugated to the BSA or ovalbumin to bind to DEC.

In another embodiment, the heavily glycosylated protein fetuin, present in fetal calf serum, can be used to evaluate glycan ligands. A fetuin binding system, based on T cell activation or endocytosis of a marker, can be developed. Specific glycosidases can be used to specifically "knock-out" glycans, and the ability of the

modified fetuin to function in the binding system evaluated. Diminishment of functional activity would indicate that the enzymatically modified sugar residue was involved in binding to DEC.

- 5 In a further aspect, the observation that the ninth and tenth lectin domains of DEC may be involved in membrane associated antibody-mediated antigen presentation, by "chaperoning" antibody into endosomes, suggests that these domains are specific for binding carbohydrates found on cell surface immunoglobulin molecules.
- 10 Another approach uses recombinant bacteriophage to produce large libraries. Using the "phage method" (Scott and Smith, 1990, Science 249:386-390; Cwirla, et al., 1990, Proc. Natl. Acad. Sci., 87:6378-6382; Devlin et al., 1990, Science, 249:404-406), very large libraries can be constructed (10^6 - 10^8 chemical entities). A second approach uses primarily chemical methods, of which the Geysen method (Geysen et
- 15 al., 1986, Molecular Immunology 23:709-715; Geysen et al. 1987, J. Immunologic Method 102:259-274) and the recent method of Fodor et al. (1991, Science 251, 767-773) are examples. Furka et al. (1988, 14th International Congress of Biochemistry, Volume 5, Abstract FR:013; Furka, 1991, Int. J. Peptide Protein Res. 37:487-493), Houghton (U.S. Patent No. 4,631,211, issued December 1986) and
- 20 Rutter et al. (U.S. Patent No. 5,010,175, issued April 23, 1991) describe methods to produce a mixture of peptides that can be tested as agonists or antagonists.

In another aspect, synthetic libraries (Needels et al., 1993, "Generation and screening of an oligonucleotide encoded synthetic peptide library," Proc. Natl.

- 25 Acad. Sci. USA 90:10700-4; Lam et al., International Patent Publication No. WO 92/00252 and U.S. Patent No. 5,382,513, issued January 17, 1995, each of which is incorporated herein by reference in its entirety), and the like can be used to screen for ligands according to the present invention.
- 30 Alternatively, assays for binding of soluble ligand to cells that express recombinant forms of a ligand binding domain or domains (preferably domains) of DEC can be

performed. As discussed in the Examples, *infra*, the presence of multiple lectin domains on DEC may contribute to the affinity and specificity of binding to glycans.

- 5 The screening can be performed with recombinant cells that express the DEC, or alternatively, using purified receptor protein, *e.g.*, produced recombinantly, as described above. For example, the ability of labeled, soluble or solubilized DEC that includes the ligand-binding portion of the molecule, to bind ligand can be used to screen libraries, as described in the foregoing references.

10

Antibodies to DEC

According to the invention, DEC produced recombinantly or by chemical synthesis, and fragments or other derivatives or analogs thereof, including fusion proteins, may be used as an immunogen to generate antibodies that recognize DEC. Such
15 antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, *infra*, a rabbit polyclonal antibody is prepared against the N-terminal amino acid sequence of DEC-205. In another, a polyclonal antibody against intact, purified, DEC-205 was generated.

20

Various procedures known in the art may be used for the production of polyclonal antibodies to DEC, or derivative or analog thereof. For the production of antibody, various host animals can be immunized by injection with the non-allogeneic DEC, or a derivative (*e.g.*, fragment or fusion protein) thereof, including but not limited
25 to rabbits, mice, rats, sheep, goats, etc. In one embodiment, the DEC or fragment thereof can be conjugated to an immunogenic carrier, *e.g.*, bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various adjuvants may be used to increase the immunological response, depending on the host species, as described above.

30

For preparation of monoclonal antibodies directed toward DEC, or fragment, analog, or derivative thereof, any technique that provides for the production of antibody molecules by continuous cell lines in culture may be used. These include but are not limited to the hybridoma technique originally developed by Kohler and
5 Milstein (1975, Nature 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In an additional embodiment of the invention, monoclonal antibodies can
10 be produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96). In fact,
15 according to the invention, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, J. Bacteriol. 159:870; Neuberger et al., 1984, Nature 312:604-608; Takeda et al., 1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for an DEC together with genes from a human antibody molecule of appropriate biological activity can be used;
20 such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce DEC-specific single chain antibodies. An additional embodiment of the invention utilizes the
25 techniques described for the construction of Fab expression libraries (Huse et al., 1989, Science 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity for DEC, or its derivatives, or analogs.

Antibody fragments which contain the idiotype of the antibody molecule can be
30 generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ fragment which can be produced by pepsin digestion of the

antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

- 5 In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, *e.g.*, radioimmunoassay, ELISA (enzyme-linked immunosorbant assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, *in situ* immunoassays (using colloidal gold, enzyme or radioisotope labels, 10 for example), western blots, precipitation reactions, agglutination assays (*e.g.*, gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by 15 detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention. For example, to select antibodies which recognize a specific epitope of DEC, one may assay generated hybridomas for a product which binds to 20 a DEC fragment containing such epitope. For selection of an antibody specific to DEC from a particular species of animal, one can select on the basis of positive binding with DEC expressed by or isolated from cells of that species of animal, and the absence of binding to DEC from other species. Binding to DEC may be detected as binding to dendritic cells that express DEC.

25

- The foregoing antibodies can be used in methods known in the art relating to the localization and activity of the DEC, *e.g.*, for Western blotting, imaging DEC *in situ*, measuring levels thereof in appropriate physiological samples, etc. The antibodies of the present invention advantageous provide for detecting and 30 enumerating human dendritic cells. Alternatively, such antibodies can be used to isolate human dendritic cells, *e.g.*, by panning. In yet another embodiment, the

antibodies of the invention can be used to target molecules to human dendritic cells. It will be recognized that this is a significant advantage, since the prior art antibody of Kraal et al. failed to recognize human DEC.

- 5 Antibodies that are targeted to DEC and participate in the activity of DEC, *e.g.*, endocytosis, can be generated. Such antibodies can be tested using the assays described *supra* for identifying ligands. In a specific embodiment, a rabbit polyclonal anti-DEC antibody targets binding of DEC, is endocytosed, and is efficiently presented to immunoglobulin-specific T cells.

10

Targeting Molecules to DEC

- The present invention advantageously provides for targeting molecules to DEC for immune modulation, *e.g.*, stimulation of T cell immunity, suppression immunity or induction of T cell anergy, and clonal deletion mechanism; trans-epithelial
- 15 transport, with delivery of a molecule across epithelium into the pulmonary circulation or intestinal circulation, or from the bloodstream into the pulmonary or intestinal lumen; and crossing the blood brain barrier. In particular, a ligand for DEC, as described *supra*, or an antibody reactive with DEC (or a DEC-binding portion thereof), as described *supra*, is conjugated to a molecule which is to be
- 20 targeted to DEC.

- Immunomodulation.* With respect to immunomodulation, the present invention provides for both stimulating T cell-mediated immune responses, particularly for vaccination, and inducing tolerance, particularly with respect to autoimmunity.

25

- Stimulation of T cell immunity can be effected by introducing an antigen, *e.g.*, a weak or poorly immunogenic antigen, conjugated to a DEC-binding moiety (ligand or antibody) into a subject, along with a factor that activates the dendritic cells that initially present antigen to the T cells. Dendritic cell activation can be
- 30 accomplished by use of an adjuvant, such as an adjuvant as described above, which has the ability to induce a generalized immune response. Alternatively, the

"vaccine" of the invention may comprise the antigen conjugated to the DEC-binding moiety and a cytokine or a lymphokine, such as granulocyte-macrophage colony stimulating factor (GM-CSF), or some other CSF. Suitable antigens for use in such a vaccine include bacterial, viral, parasite, and tumor antigens.

5

Alternatively, the present invention provides for inducing tolerance. Tolerance is desirable to avoid detrimental immune responses, in particular, autoimmunity and allograft rejection. Presentation of antigen by non-activated dendritic cells, *e.g.*, in the skin and T cell areas of the lymphoid organs, induces T cell anergy, and
10 possibly causes destruction of the responder clone. Thus, in one embodiment, tolerance is induced by administering an antigen modified by conjugation with a DEC-binding moiety under conditions that promote dendritic cell quiescence, *e.g.*, in the absence of an infection, without adjuvant, using pyrogen-free pharmaceutical carriers, and in the absence of additional lymphokines or cytokines.

15

It is further believed that high level expression of DEC may act as a tolerizing influence. Accordingly, the invention further relates to introducing recombinant dendritic cells, or cell recombinantly modified to express both DEC and MHC Class II, into a subject, along with antigen conjugated to a DEC-binding moiety.
20 Alternatively, the *dec* gene can be targeted to appropriate cells *in vivo*, for gene therapy.

In a further embodiment, tolerance can be induced through the clonal deletion mechanism. In particular, antigen conjugated with a DEC-binding moiety can be
25 introduce into a subject, preferably directly into the thymus, either by targeting or physical injection, for processing and presentation by the thymic epithelium and medullary dendritic cells. This processing and presentation step is believed to be involved in the selection process to eliminate autoreactive T cells, *i.e.*, clonal deletion. In a further aspect, the level of expression of DEC may be manipulated,
30 *e.g.*, by introducing additional *dec* genes into the thymic epithelium and medullary dendritic cells.

Attractive candidates for conjugation with a DEC-ligand to induce tolerance, T cell anergy, or clonal deletion include, but by no means are limited to, allergenic substances, autoantigens such as myelin basic protein, collagen or fragments thereof, DNA, nuclear and nucleolar proteins, mitochondrial proteins, pancreatic β -cell proteins, and the like (*see* Schwarz, 1993, In *Fundamental Immunology, Third Edition*, W.E. Paul (Ed.), Raven Press, Ltd.: New York, pp. 1033-1097).

Trans-Epithelial Migration. In another embodiment, a molecule can be targeted for trans-epithelial migration by conjugating it with a DEC-binding moiety. In one aspect of the invention, the invention provides for targeting a therapeutic molecule for absorption across lung or intestinal epithelium. Thus, the invention provides for delivering an aerosolized therapeutic agent by inhalation, *i.e.*, by pulmonary administration of the drug. In another aspect, the invention provides for delivery of a therapeutic agent by DEC-mediated absorption across the small intestine. In particular, the invention advantageously provides for absorption, or more accurately, trans-mucosal migration, of hydrophilic molecules, which are usually not as easily absorbed as hydrophobic molecules. This aspect of the invention takes advantage of the presence of DEC on the apical (or luminal) surface of the epithelial cells.

In another aspect of the invention, the presence of DEC on the basolateral surface of the epithelial cells provides a route for transport of a molecule conjugated to a DEC-ligand from the bloodstream into the lumen of the lung or the small intestine. This delivery route can be very important for administration of an acid labile, hydrophilic therapeutic agent to the intestines. Such a drug cannot be ingested, as the acid conditions present in the stomach would result in its destruction; transport of such a drug from the bloodstream to the lumen of the intestines would not readily occur spontaneously, since a hydrophilic agent does not have a significant partition coefficient across cell membranes. In specific embodiments, the present invention provides for administration of chemotherapeutic agents and antibiotics, particularly anti-parasite drugs, by conjugating them to a ligand for DEC.

administering the agent parenterally, preferably intravenously, such that the drug is targeted for transport from the basolateral surface of the intestinal epithelium to the luminal surface.

- 5 In the same way, a therapeutic agent may be targeted for delivery from the bloodstream to the airways of the lung by targeting the DEC receptor on the basolateral surface of the lung epithelium. Such a delivery system would be particularly advantageous for delivery of drugs to individuals with impaired lung capacity, *e.g.*, who cannot inhale adequately, and thus, for whom administration via
10 the bloodstream is indicated. Such lung impairments include, but are not limited to, pneumonia, emphysema, lung cancer, adult respirator distress syndrome, dyspnea, hemoptysis, chronic obstructive pulmonary disease (COPD), fibrogenic dust diseases, pulmonary fibrosis, organic dust diseases, chemical injury, smoke injury, thermal injury (burn or freeze), asthma (allergy, bronchoconstriction, other
15 causes of asthma, *e.g.*, irritants), hypersensitivity pneumonitis, Goodpasture's Syndrome, pulmonary vasculitis, and immune complex-associated inflammation. Thus, the invention provides for administration of antibiotics, anti-inflammatory agents, complement inhibitors (*e.g.*, complement receptor 1 [CD35]), and the like for trans-epithelial migration into the lumen of the lung.

20

- Trans-Blood Brain Barrier Migration.* In still another embodiment, a molecule targeted for the brain can be conjugated to a DEC-binding moiety. The molecule would then bind to DEC found in the capillaries of the brain, which are believed to promote trans-blood brain barrier transport or migration. Presently, there are few
25 or no generally effective mechanisms for directing molecules across the blood brain barrier. Such molecules for transport across the blood brain barrier include, but are not limited to, neurotrophic factors (brain-derived neurotrophic factor, NT-3, NT-4, ciliary neurotrophic factor), growth factors (*e.g.*, nerve growth factor), and the like: antibiotic or antiviral agents, for incipient infections of the brain; and vectors for
30 gene therapy.

Targeting Vectors for Gene Therapy. In yet another embodiment, the present invention provides ligands for targeting DNA vectors to cells that express DEC, in particular, dendritic cells, epithelial cell of the thymus, small intestine, and lung, and brain capillaries. Accordingly, a DNA vector, such as a viral vector, can be modified by conjugation with a DEC ligand for targeting to cells that express DEC. Examples of DNA virus vectors include, but are not limited to, a defective herpes virus 1 (HSV1) vector (Kaplitt et al., 1991, Molec. Cell. Neurosci. 2:320-330), an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al. (1992, J. Clin. Invest. 90:626-630), a defective adeno-associated virus vector (Samulski et al., 1987, J. Virol. 61:3096-3101; Samulski et al., 1989, J. Virol. 63:3822-3828), as well as a papillomavirus vector, Epstein Barr virus (EBV) vector, and the like. The viral particles can be modified to include a ligand for DEC, *e.g.*, by chemically cross-linking a DEC ligand to the virus.

Alternatively, the vector can be introduced *in vivo* by lipofection. Synthetic cationic lipids designed to limit the difficulties and dangers encountered with liposome mediated transfection can be used to prepare liposomes for *in vivo* transfection of a gene encoding DEC (Felgner, et. al., 1987, Proc. Natl. Acad. Sci. U.S.A. 84:7413-7417; *see* Mackey, et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:8027-8031)). The use of cationic lipids may promote encapsulation of negatively charged nucleic acids, and also promote fusion with negatively charged cell membranes (Felgner and Ringold, 1989, Science 337:387-388). The use of lipofection to introduce exogenous genes into the specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. Accordingly, the present invention advantageously provides for targeting a gene for dendritic cells and thymic epithelium by conjugating a DEC-ligand to a liposome vector. Lipids may be chemically coupled to other molecules for the purpose of targeting (*see* Mackey, et. al., 1988, *supra*). Targeted antibodies or glycans could be coupled to liposomes chemically.

It is also possible to introduce the vector *in vivo* as a naked DNA plasmid, preferably by using a DEC ligand as a vector transporter (see, *e.g.*, Wu et al., 1992, J. Biol. Chem. 267:963-967; Wu and Wu, 1988, J. Biol. Chem. 263:14621-14624; Hartmut et al., Canadian Patent Application No. 2,012,311, filed March 15, 1990).

5

The present invention will be better understood by reference to the following Examples, which are provided by way of exemplification and not limitation.

10 EXAMPLE 1: DEC-205, A 205 kDa PROTEIN ABUNDANT ON MOUSE
 DENDRITIC CELLS AND THYMIC EPITHELIUM THAT
 IS DETECTED BY THE MONOCLONAL ANTIBODY
 NLDC-145: PURIFICATION, CHARACTERIZATION, AND
 N-TERMINAL AMINO ACID SEQUENCE

15

This Example describes the purification and biochemical characterization of the antigen recognized by monoclonal antibody NLDC-145 (Krall et al., 1986, J. Exp. Med. 163:981). We refer to the protein as DEC-205, in view of its abundant expression by Dendritic and thymic Epithelial Cells, and the observed molecular
20 mass. The protein has been purified at a scale that permits direct biochemical study. The antigen proves to be an integral membrane glycoprotein with a mildly alkaline (pI 7.5) and an electrophoretic molecular mass of 205 kDa, not 145 kDa, as originally reported (Kraal et al., *supra*). About 7 kDa of the mass is contributed by covalently-bound carbohydrates. A panel of plant lectins was used to gain
25 preliminary information on the structures of these glycans. The glycans were then subjected to a variety of exoglycosidase digestions and fluorophore-assisted carbohydrate electrophoresis (FACE) (Jackson, 1990, Biochem. J. 270:705; Jackson and Williams, 1991, Electrophoresis 12:94; Jackson, 1993, Biochem. Soc. Trans. 21:121; Jackson, 1994, Anal. Biochem 216:243). Eight distinct but related
30 biantennary N-linked glycan structures were resolved. These variants differed at their termini, but were based on two fucosylated trimannosyl chitobiose core structures (with and without a bisecting GlcNAc). O-linked glycans were not detected. The amino terminus of the protein is not blocked, and the sequence of its

first 25 amino acids is not significantly homologous or similar to any known protein. Two new polyclonal antibodies are described, one raised to the N-terminal peptide sequence, the second to the intact purified protein. On immunoblots, both of these polyclonals recognize a 205 kDa band, which can be specifically depleted
5 by preclearing extracts with NLDC-145.

Materials and Methods

Purification of NLDC-145 and preparation of immunoaffinity resins-- NLDC-145 (rat IgG2a) ascitic fluids were prepared in normal, 6-8 week old, non-SPF, CD2
10 (BALB/c x DBA/2 F1) female mice (Trudeau Institute) as described (North and Izzo, 1993, J. Exp. Med. 177:1723). The monoclonal was purified by sequential chromatography on immobilized Protein A and Protein G (Pierce). Both the monoclonal and nonspecific rat IgG2a (Zymed) were coupled to resins by reductive amination (AminoLink, Pierce).

15

Immunoprecipitation -- Bone marrow dendritic cells (BMDC) were prepared from proliferating marrow precursors. as described by Inaba et al. (1992, J. Exp. Med 176:1693). Eight days after the cultures were initiated, 4.8×10^7 BMDC were cultured for 1 h in 10 ml of methionine- and cysteine-free medium. Labeling was
20 initiated by adding 1 mCi of (35 S)methionine-cysteine (ICN), and cells were collected after 4 h of culture. BMDC were lysed by resuspending them in 700 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 6.8, 1% Nonidet P-40 (Calbiochem), 50 mg/ml BSA (Intergen), with a mixture of protease inhibitors: 5 mM EDTA, 0.5 mg/ml Pefabloc SC (Boehringer Mannheim), 100 mg/ml PMSF, 5 mg/ml aprotinin,
25 5 mg/ml pepstatin A and 10 mg/ml leupeptin (the latter 4 inhibitors from Sigma)). Lysates were precleared with 20 mg of rat IgG (Jackson ImmunoResearch), 10 ml of FCS, and 100 ml of packed immobilized Protein G (Pierce). The supernatant was precleared a second time with 100 ml of Protein G, for 1 h. The precleared lysate was divided into 100 ml aliquots. Proteins in two aliquots (6.9×10^6 BMDC
30 equivalents) were adsorbed to 50 ml of packed, washed immunoaffinity resin (either NLDC-145 or rat IgG2a), rotating 1 h. Washes were performed as

described (Fireston and Winguth, 1990, Methods Enzymol. 182:688). Proteins were analyzed by SDS-PAGE in 10% acrylamide minigels.

- Immunoblotting*-- SDS-PAGE was performed in 8% acrylamide minigels, 1.5 mm thick. Transfer to nitrocellulose (BA-85, Schleicher and Schuell) was performed at 30 constant volts overnight at 4°C. Filters were blocked in PBS containing 3% (w/v) nonfat dry milk and 0.1% Tween 20 for 1 h at room temperature with shaking. Incubation with primary antibodies (0.1-10 µg/ml of purified IgG, ascites or serum diluted 1:1000, or hybridoma supernatant diluted 1:1) was performed in heat-sealed bags for 1 h at room temperature. Filters were washed, then immunostaining was visualized with peroxidase-conjugated F(ab')₂ donkey anti-rat or anti-rabbit IgG (Jackson), followed by enhanced chemiluminescence (Amersham).
- Purification of DEC-205 from thymi*-- The strategy is summarized in Figure 2. Thymi were removed from 50 outbred CD-1 Swiss mice (Taconic) per preparation. Thymi were placed into 50 ml of ice-cold PBS containing 200 mg/ml PMSF and 5 mM EDTA to remove blood, and washed once with the same buffer. Washed organs could be frozen at -20°C. All subsequent purification steps were performed at 0-4°C. Thymi were transferred to a 40 ml Dounce homogenizer (Kimble/Kontes), and resuspended in 30 ml of hypotonic lysis buffer (10 mM Tris-HCl, pH 6.8, with a mixture of protease inhibitors: 5 mM EDTA, 100 mg/ml PMSF, 4 mg/ml aprotinin, 0.5 mg/ml Pefabloc SC, 4 mg/ml pepstatin A, 10 µg/ml leupeptin). Organs were homogenized with 20 strokes of the loose (0.2 mm clearance) pestle, then 20 strokes of the tight (0.1 mm clearance) pestle. The suspension was left on ice for 20 min, then re-homogenized with an additional 20 strokes of the tight pestle. Nuclei and debris were pelleted by low speed centrifugation (1200 x g, 5 min, 4°C). The turbid "postnuclear" supernatant was collected, and the nuclear pellet was washed with 5-8 changes of 15 ml hypotonic lysis buffer, until the supernatant was nearly clear. Supernatants from each wash were pooled. To collect membranes, pooled postnuclear supernatant was

centrifuged at 100,000 x g for 1 h at 4°C (RC-28S centrifuge, F28/36 rotor, Du Pont-Sorvall). Proteins in the membrane pellet were extracted into 5 ml of hypotonic lysis buffer containing 0.5% (8.3 mM) NP-40. The membrane extract was clarified by a second one-hour, 100,000 x g centrifugation.

5

Clarified membrane extract was precleared by passage over a nonspecific rat IgG column. Nonadsorbed fractions from the preclearing column were pooled, then applied to the NLDC-145 affinity column. Washes were performed in 2 steps: 6 ml (3 bed volumes) of wash-1 (hypotonic lysis buffer with 0.5 M NaCl, without
10 NP-40, substituting 0.5% (17 mM) n-octyl glucoside (Boehringer Mannheim)), then 10 ml of wash-2 (wash-1 without added NaCl). The column was eluted with at least 5 bed volumes of 50 mM glycine-NaOH, pH 11, 0.5% n-octyl glucoside, reducing the maximum flow rate to 10 ml cm⁻² hr⁻¹. The pH of eluted fractions (1 ml) was adjusted to 7 with 20-30 ml of 2 M glycine-HCl, pH 2. Peak eluates were
15 pooled and concentrated to <1 ml by ultrafiltration in Centricon-100 units (Amicon) that had been pre-coated with 0.1% SDS, to reduce nonspecific losses to the plastic. Typically, 70-150 µg of DEC-205 could be obtained from 50 thymi.

Isoelectric focusing-- Isoelectric focusing was performed in thin (0.75 mm) slab
20 gels, under denaturing conditions (5.5% acrylamide gels containing 8 M urea, 4% total Ampholine (2:1 ratio of pH 3.5-10 and 5-7, Pharmacia), 0.67% NP-40, 10% glycerol). Samples were focused at 400 constant volts overnight, for a minimum of 6000 volt-hours, at which time the current was less than 1 mA. Lanes were either silver-stained or cut into 0.5 cm sections and eluted into degassed dH₂O for pH
25 gradient measurement.

Detection of glycans-- DEC-205, transferrin (positive control) and creatinase (negative control) were blotted onto nitrocellulose as before. Glycoconjugates were converted to digoxigenin (DIG)-labeled hydrazones after mild nonselective
30 periodate oxidation of vic-diols to aldehydes. Staining patterns were visualized

with an anti-DIG antibody conjugated to alkaline phosphatase (First CHOice, Boehringer Mannheim).

Chemical deglycosylation-- Two 100 μ l samples each of DEC-205 (40 μ g) and
5 apotransferrin (100 μ g, positive control) were transferred into 0.1% trifluoroacetic acid, 0.05% SDS by G-25 SF spin chromatography, then were lyophilized to dryness. Cleavage was performed with anhydrous trifluoromethanesulfonic acid (Sojar et al., 1987, Methods Enzymol. 138:341) (Oxford GlycoSystems). Polypeptides were separated from cleavage products and excess reagents by TCA
10 precipitation, followed by electrophoresis in 8% acrylamide minigels, adjacent to untreated controls.

Enzymatic deglycosylation-- Peptide-N-glycosidase F from *Flavobacterium meningosepticum* (PNGase F, Boehringer Mannheim) was used to cleave
15 asparagine-linked glycans (Tarentino et al., 1985, Biochem. 24:4665). Aliquots (10 μ g per eventual gel lane) of DEC-205 were denatured by boiling for 5 min in the presence of 0.1% SDS. After cooling on ice and brief spinning in a microfuge to collect liquid, 1/5 volume of 5X PNGase buffer (250 mM sodium phosphate, pH 7.0, 50 mM EDTA, 2.5% NP-40, 5% 2-mercaptoethanol) was added, then 1 unit of
20 PNGase F (5 μ l). Reactions were incubated overnight at 37°C, then were terminated by adding 1/4 volume of 4X nonreducing SDS-PAGE sample buffer, and boiling for 5 min.

Lectin blotting-- Several digoxigenin-labeled plant lectins (Boehringer Mannheim)
25 were used to stain electroblotted DEC-205 and appropriate positive and negative control glycoproteins. The lectins, their specificities and the concentrations used for staining are summarized in Table 1.

Exoglycosidase digestions and FACE analysis-- N-linked oligosaccharides were
30 released from DEC-205 with PNGase F and labeled with the fluorophore ANTS (8-aminonaphthalene-1,3,6-trisulfonic acid) (Jackson, 1990, *supra*; Jackson and

Williams, *supra*; Jackson, 1993, *supra*; Jackson, 1994, *supra*). Recombinant exoglycosidases were from Glyko. Electrofluorograms were visualized on an SE1000 FACE workstation (Glyko).

- 5 *Amino acid sequencing*-- DEC-205 was electrophoresed in multiple lanes of 1.5 mm thick 4% minigels prepared using Duracryl (Millipore). Gels were blotted onto polyvinylidene difluoride (PVDF, Bio-Rad). After transfer, filters were soaked for 1 min in 1% acetic acid, stained for 2 min in 0.1% Ponceau S, then were destained briefly in dH₂O. Bands at 205 kDa were excised and submitted for
10 analysis. The N-terminal sequence was aligned to all current databases on the BLAST Internet servers (NCBI, National Library of Medicine, NIH), running the program BLASTP (Altschul et al., 1990, J. Med. Biol. 215:403).

- Polyclonal antibodies to intact DEC-205*-- Two New Zealand White rabbits
15 (Hazelton) were injected 6 times with the 205 kDa bands cut from Coomassie-stained, 1.5 mm thick, 4% Duracryl SDS-PAGE gels. Doses ranged from 40-70 µg of stained protein per animal, per injection (4-6 slices), and were given every 3 weeks, with test bleeds (about 15 ml of serum) taken 2 weeks post-injection. For the first injection, slices were emulsified in Complete Freund's adjuvant (CFA) and
20 injected intradermally into multiple sites on the back. Incomplete Freund's (IFA) was the adjuvant for boosts. Responses were monitored by Western blotting crude thymic membrane extracts with graded doses of serum. Animals were boosted further with the unfractionated eluate from the immunoaffinity column, i.e., soluble protein rather than gel slices. Four boosts, averaging 50 µg per injection, were
25 given to both rabbits. IgG fractions were prepared by Protein A chromatography.

- Polyclonal antibodies to the N-terminal peptide*-- The hapten-coupling strategy focused on the lone cysteine at residue 19 (Figure 6A). Peptide N1 (SESSGNDPFTIVHENTGKC) (SEQ ID NO: 2) was coupled to keyhole limpet
30 hemocyanin (KLH) and ovalbumin (OVA) using maleimide chemistry (Imject, Pierce). An average of about 250 peptides were conjugated to each molecule of

KLH, and about 6 peptides per molecule of OVA. The KLH-peptide conjugate was divided into aliquots of 400-500 μ g each, and was injected eight times into two New Zealand White rabbits (200-250 μ g per injection), again emulsifying into CFA for the initial immunization and IFA for boosts. To remove any anti-KLH reactivity from the sera, they were precleared on a KLH-cysteine column. Anti-peptide antibodies were isolated on a peptide-OVA column, where the peptide was coupled to an irrelevant carrier.

Results

10 *NLDC-145 immunoprecipitates and Western blots an antigen of 205 kDa, not 145 kDa--* To determine the molecular mass of the NLDC-145 antigen, DCs generated from proliferating bone marrow progenitors *in vitro* (Inaba et al., *supra*). This method provided abundant NLDC-145 (+) DCs in high purity. On day 8 of culture, the DCs were metabolically labeled with [35 S]methionine-cysteine. Extracts
15 were immunoprecipitated using either immobilized NLDC-145 or control nonspecific rat IgG2a. Autoradiography of the precipitates after reducing SDS-PAGE (Figure 1A) revealed that NLDC-145 bound a single specific band with an apparent mass slightly greater than 200 kDa (myosin marker), not 145 kDa, as originally reported (Kraal et al., *supra*). Numerous nonspecific bands were also
20 visible in both lanes, including a prominent band at 45 kDa, presumably G-actin (Fosman, 1976, *Handbook of Biochemistry and Molecular Biology, Volume 1: Proteins*, CRC Press: Cleveland, Ohio).

To verify this measurement, NLDC-145 was used as the probe in a Western blot.
25 Five thymi from 8 week-old BALB/c mice were homogenized in 2 ml of the same lysis buffer used for immunoprecipitation. Graded doses of clarified thymic extract were electrophoresed under nonreducing and reducing conditions, and blotted to nitrocellulose. NLDC-145 bound a single major band (Figure 1B) that co-migrated with the *prestained* myosin marker at 205 kDa, confirming the estimate made by
30 immunoprecipitation (above). Under nonreducing conditions (Figure 1B, left filter), as few as 7×10^{-4} thymic equivalents could be clearly visualized with this

dose of monoclonal NLDC-145 IgG. However, after reduction of disulfides in the crude extract with mercaptoethanol (right strip), the mAb failed to stain, even at the highest dose of lysate. We concluded that the antigen recognized by NLDC-145 has a mass of 205 kDa, and that the epitope detected by the mAb requires an
5 intramolecular disulfide bond.

Purification of DEC-205-- The epithelial cells of the thymic cortex express the antigen abundantly (Kraal et al., *supra*), even more abundantly than the less-numerous DCs in the thymic medulla. Therefore, a scheme to isolate the protein
10 from thymi was developed (Figure 2). Briefly, thymi were homogenized under hypotonic lysis conditions, in the presence of a "cocktail" of high doses of 6 protease inhibitors. Membranes were isolated and extracted with NP-40. After clarification, the extract was precleared on a rat IgG column, then chromatographed on the NLDC-145 column.

15

SDS-PAGE analysis of purified, concentrated DEC-205 (Figure 3A) revealed a predominant 205 kDa band and only trace contaminant bands on Coomassie staining. Silver staining exposed a large number of lower molecular mass contaminants, dictating the need for further purification by preparative
20 electrophoresis before amino acid sequencing.

In an effort to follow step yields during the purification process, key fractions were diluted to the same volume (the volume of the post-nuclear supernatant), and immunoblotted (Figure 3B). Because the fractions were isovolumic, staining
25 intensities on the filter should reflect the relative concentrations of DEC-205 at each phase of the separation. Roughly 30% of the protein appeared to be lost to the nuclear pellet (lane 1) in the first step, hypotonic lysis. Another 10% or so (lane 3) failed to sediment with the membrane pellet (lane 4) during the first high-speed centrifugation. A constant small fraction -- perhaps 5-10% cumulatively --
30 passed through the NLDC-145 column, in both early and late nonadsorbed fractions

(lanes 6 and 7). The cumulative yield in the final eluate (lane 8) was therefore about 50%.

When the eluate was intentionally overloaded at five times the isovolumic concentration (lane 10), a "ladder" of at least 6 smaller, minor bands was clearly and consistently seen. All of these bands must contain the NLDC-145 epitope. They ranged down in rather orderly fashion (i.e., not a continuous "smear") to a fairly intense 80 kDa component. Careful inspection of earlier fractions (in particular, lanes 2, 3, 4, and 8) revealed that these minor bands were present from the earliest stages in the isolation. Presumably, these bands were produced by proteolytic degradation of the intact 205 kDa protein.

DEC-205 is an integral membrane protein with an isoelectric point of 7.5-- To determine whether the 205 kDa protein was an integral membrane protein, thymic membrane pellets (prepared as in Figure 2) were resuspended in: (1) hypotonic lysis buffer containing 0.5% NP-40 (as usual); (2) the same buffer containing 1 M KCl instead of detergent; or (3) 100 mM Na₂CO₃ pH 11.5, containing all 6 protease inhibitors used in routine purifications. After one hour of gentle mixing, suspensions were clarified (100,000 x g, 60 min, 4°C), and supernatants were collected. Precipitates from the high-salt and high-pH extractions were then resuspended in hypotonic lysis buffer with 0.5% NP-40, and the extraction and clarification steps were repeated. A Western blot of the five extracts generated this way (Figure 4A) revealed that DEC-205 could not be released from the membrane pellet under conditions of either high ionic strength (lane 2) or extreme pH (lane 3). Detergent was required for its solubilization (lanes 1, 4 and 5). DEC-205 is therefore an integral membrane protein (Fujiki et al., 1982, J. Cell Biol. 93:97).

Isoelectric focusing was performed in slab gels under denaturing conditions with silver staining. A relatively homogeneous isoelectric point was observed at pH 7.5 (Figure 4B). A sharply focused central band at that pH was consistently bordered by a narrow "fringe" of fainter staining, extending from pH 7.4 to pH 7.6.

DEC-205 is a glycoprotein, bearing heterogeneous N-linked glycans -- To determine whether DEC-205 was glycosylated, purified 205 kDa protein was electrophoresed on a gel which also contained samples of transferrin (a known glycoprotein) and creatinase (a known nonglycoprotein), and was electroblotted to
5 nitrocellulose. Filters were oxidized with sodium *meta*-periodate at room temperature, nonselectively converting vicinal diols in carbohydrates to aldehydes. A digoxigenin (DIG)-tagged hydrazide was applied to the filter, converting the aldehydes to DIG-hydrazones. The filter was blocked, and then covalently-bound DIG was detected by staining with an anti-digoxigenin antibody conjugated to
10 alkaline phosphatase. The staining pattern (Figure 5A) revealed that DEC-205 (lane 2) is a glycoprotein, like transferrin (lane 1).

To determine how much of the apparent molecular mass was contributed by glycans, the purified protein was chemically deglycosylated. Anhydrous
15 trifluoromethanesulfonic acid (TFMSA) does not attack the primary structure of proteins, yet hydrolyzes both asparagine-linked and serine/threonine-linked glycans at their points of attachment to amino acid sidechains (Sojar et al., *supra*; Dabich et al., 1993, Biochem. Biophys. Acta 1164:47). Upon TFMSA treatment (Figure 5B), both apotransferrin (lane 2) and DEC-205 (lane 4) exhibited increased
20 electrophoretic mobility compared to untreated samples (lanes 1 and 3). Linear regression analysis of the migratory distances of the treated samples revealed that, as expected, deglycosylated apotransferrin lost 5 kDa in apparent molecular mass (MacGillivray et al., 1983, J. Biol. Chem. 258:3543), while DEC-205 lost roughly 7 kDa. This 7 kDa shift was consistent with the removal of two to three complex-
25 type N-linked glycans from the 205 kDa protein. An uncertain number of smaller O-linked glycans might also have been removed.

To begin to define the types of carbohydrate residues present on the protein, blotted purified material was probed with a panel of digoxigenin-labeled plant lectins.
30 Asparagine-linked glycans were removed from an aliquot of DEC-205 by treating it with peptide N-glycosidase F (PNGase F). Treated and untreated protein was

blotted to nitrocellulose along with positive and negative control glycoproteins. After confirming transfer by staining the filters with Ponceau S (not shown), membranes were blocked and stained with DIG-lectins, used at the concentrations listed in Table 1.

5

TABLE 1

Table 1: Staining of electroblots with digoxigenin-labeled plant lectins

Lectin	Source	Specificity	Concentration ($\mu\text{g/ml}$)	Staining on	Staining on
				PNGaseF-treated DEC-205	undigested DEC-205
SNA	<i>Sambucus nigra</i>	NANA α 2-6Gal/GalNAc	1	(-)	(-)
MAA	<i>Maackia amurensis</i>	NANA α 2-3Gal	5	(-)	(-)
PNA	<i>Arachis hypogaea</i>	Gal β 1-3GalNAc	10	(-)	(-)
DSA	<i>Datura stramonium</i>	Gal β 1-4GlcNAc, GlcNAc-Ser/Thr	1	(-)	strong (+)
AAA	<i>Aleuria aurantia</i>	L-Fuc α 1-6GlcNAc	1	(-)	strong (+)
GNA	<i>Galanthus nivalis</i>	Man α 1-3Man (α 1-3 > α 1-6 > α 1-2)	1	(-)	very weak (+) at 200 kDa

Lectin staining patterns (Table 1) showed that the N-linked glycans lacked N-acetylneuraminic acid in either of its two most common linkages to galactose, α 2-6 and α 2-3, since the protein failed to bind lectins SNA and MAA, respectively. The core disaccharide of O-linked glycans was not present in unsubstituted form, since PNA did not bind. Pretreatment of the protein with neuraminidase did not render it stainable with PNA (not shown), so any O-glycans present were not capped with sialic acid. If present, they are few in number, since selective removal of N-linked glycans with PNGase F reduced the protein's apparent mass by 7 kDa (not shown), just as nonselective chemical deglycosylation did. Undigested DEC-205 stained intensely with DSA, and staining was ablated by PNGase F digestion. Thus, one or more of the N-glycans terminates with Gal β 1-4GlcNAc. Terminal fucose linked α 1-6 to GlcNAc is also present on at least one of the N-glycans, since the undigested protein stained strongly with AAA. Lectin GNA weakly stained a band with a mobility slightly greater than the DSA (+) and AAA (+) bands. Presumably

this 200 Kda band, containing high-mannose N-linked glycans, represented a subpopulation of newly-synthesized molecules which had not yet undergone oligosaccharide processing reactions in the Golgi complex.

- 5 The structures of the N-linked glycans on DEC-205 were further defined by fluorophore-assisted carbohydrate electrophoresis (FACE). PNGase F released 8 different N-linked glycan structures from DEC-205, with electrophoretic migrations ranging from 5.1 to 10.1 glucose units (Figure 5C). The glycan yield was too low to permit excision and sequencing of each of the 8 individual bands, so the mixture
- 10 was subjected to analysis with exoglycosidases (Figure 5D). Digestion with α -galactosidase (lane 2) simplified the pattern, indicating that some of the glycans terminate with Gal α 1-(1 or 2)Gal. Addition of NANase III (lane 3, specific for α 2-3, α 2-6 and α 2-8 linked N-acetylneuraminic acid) simplified the pattern still further (loss of sialic acid reduces band mobility), demonstrating that some of the
- 15 glycans terminate with sialic acid. Neither NANase I (selective for α 2-3 linkages) nor NANase II (selective for both α 2-3 and α 2-6 linkages) altered the pattern of bands (not shown). This was consistent with the lack of staining by lectins SNA and MAA (above), and demonstrated that the terminal sialic acid present on certain DEC-205 glycans is linked α 2-8. Further treatment with β -galactosidase (lane 4)
- 20 produced a loss of two galactoses (consistent with DSA staining and a biantennary structure), and reduced the complexity of the pattern to a doublet. On addition of β -N-acetylhexosaminidase to the enzyme mixture (lane 5), the lower band of the doublet released 2 GlcNAc to yield a band characteristic of a fucosylated trimannosyl chitobiose core. However, the upper band resisted digestion,
- 25 suggesting the presence of either a "bisecting" GlcNAc linked to a trimannosyl core between the antennas, or possibly a branching fucose. The bisecting GlcNAc was revealed by the fact that the upper band could be at least partially digested (2-fold decrease in fluorescence intensity) when the enzyme concentration was doubled (lane 6). Further digestion with α -mannosidase (lane 7) was incomplete, mostly
- 30 releasing a single mannose (major band), but showed the beginning of release of a band that co-migrated with the fucosylated mannosylchitobiose core structure (lane 9, "FC"). Doubling the amount of α -mannosidase and adding an α -fucosidase

specific for fucose linked α 1-2,-3,-4 and -6 to GlcNAc (lane 8) led to essentially complete digestion, releasing a band that co-migrated with the non-fucosylated mannosylchitobiose core (lane 9, "C").

- 5 In combination, lectin staining and FACE analysis demonstrated that DEC-205 contains biantennary N-linked glycans with two kinds of fucosylated core structures, one with a bisecting GlcNAc, one without (Figure 5E). Further heterogeneity is introduced at the outer ends of these structures, which terminate with either α -linked galactose, β 1-4 linked galactose or α 2-8-linked sialic acid, in a
10 total of 8 different permutations.

N-terminal amino acid sequence, and polyclonal antibodies to the N-terminal peptide and intact DEC-205-- Purified DEC-205 was submitted for amino-terminal sequencing, and the first 25 residues were identified unambiguously (Figure 6A).

- 15 When the sequence was aligned against all available protein sequence databases, no significant homologies were found. In order to verify that the 205 kDa protein we had purified was the antigen recognized by NLDC-145, a 19-residue synthetic N-terminal peptide (Figure 6A, first 19 amino acids) was synthesized, purified, coupled to keyhole limpet hemocyanin, and injected into a pair of rabbits. Peptide-
20 reactive antibodies from hyperimmune sera were purified on an affinity resin prepared by coupling the peptide to the irrelevant carrier ovalbumin. In parallel, purified DEC-205 was injected into a second pair of rabbits, and hyperimmune IgG was purified by Protein A chromatography. On immunoblots, both polyclonals stained a 205 kDa band in crude extracts, like NLDC-145 (Figure 6B, 'extract'
25 lanes). The affinities of both polyclonals for the blotted 205 kDa protein were roughly 100 times higher than the monoclonal. Here, 0.1 μ g/ml of either polyclonal gave a staining intensity comparable to that obtained with 10 μ g/ml of NLDC-145 IgG. Both polyclonals bound "ladders" of minor bands, similar to those seen with the mAb. Staining of the 205 kDa band was specifically ablated
30 when the extracts were precleared with immobilized NLDC-145 (Figure 6B, 'depleted' lanes), but was restored on proteins eluted from the preclearing resin

(Figure 6B, 'eluate' lanes), confirming that the correct protein had been purified and sequenced.

Discussion

- 5 A purification method based on immunoaffinity chromatography was used to isolate the antigen bound by the mAb NLDC-145, an antigen which was reported to be expressed at high levels by murine dendritic cells and thymic epithelium (Kraal et al., *supra*). As a protein source, whole murine thymi were lysed rather than attempting the daunting task of purifying large numbers of DCs. The isolated
- 10 protein was about 95% pure, and was obtained with a yield in the hundred-microgram range, sufficient for N-terminal amino acid sequencing and basic biochemical studies. It would have required approximately 10^9 - 10^{10} DCs to provide a comparable amount of protein.
- 15 The electrophoretic molecular mass of the protein was consistently 205 kDa. In the original report by Kraal et al (*supra*), NLDC-145 was used to immunoprecipitate detergent extracts from surface-iodinated low-density lymph node cells. Only a single serine protease inhibitor, 1 mM PMSF, was present in the lysis buffer. A single predominant labeled protein was bound, with an apparent molecular mass of
- 20 145 kDa under both reducing and nonreducing electrophoresis conditions, leading the authors to append the number "145" to the clone's name. When Puré et al. (1990, J. Exp. Med. 172:1459) attempted to reproduce the immunoprecipitation, they prepared detergent extracts of (35 S)methionine-labeled cultured epidermal DCs (Langerhans cells), using a lysis buffer that contained multiple serine protease
- 25 inhibitors (1 mM PMSF, 1 μ g/ml aprotinin and 0.1% DIFP). They resolved a protein with an apparent molecular mass of > 200 kDa under reducing conditions.

In this Example, immunoprecipitation of the antigen from bone marrow dendritic cells in the presence of high doses of inhibitors directed not only to serine

30 proteases, but also to sulhydryl, aspartic and metalloproteases, yielded a protein of an apparent mass of > 200 kDa, in agreement with Puré et al. The mAb could be

used to stain Western blots under nonreducing conditions, and measured a mass of 205 kDa independently by this method. The protein that eluted from the NLDC-145 immunoaffinity column had a mass of 205 kDa, and the amino terminus of this protein revealed a sequence with no significant homology or similarity to any other protein currently in the databases. In order to prove that the 205 kDa purified protein was the antigen detected by NLDC-145, polyclonal antibodies to the N-terminal peptide and to the intact purified protein were prepared. Both polyclonals stained a 205 kDa band on immunoblots. This staining could be eliminated by pretreating extracts with NLDC-145, demonstrating that the correct protein had been purified and sequenced. Thus, the 205 kDa protein purified here is the authentic antigen recognized by the NLDC-145 monoclonal antibody. We believe that the lysis conditions employed by Kraal et al. (*supra*), with minimal antiproteolytic coverage, may have permitted limited degradation of the protein.

We propose the name "DEC-205" for the protein, to indicate its high-level expression by Dendritic and thymic Epithelial Cells, and to revise the prior estimate of its electrophoretic molecular mass. DEC-205 is an integral membrane glycoprotein, bearing 2-3 biantennary complex-type N-linked glycans that comprise about 7 kDa of the overall electrophoretic molecular mass. These glycans are built on two different core structures, and vary further at their termini, to produce 8 variants, some of which contain sialic acid. Nevertheless, on electrofocusing, the isoelectric point of DEC-205 is slightly alkaline (pH 7.5), suggesting that the protein may be relatively rich in basic residues. The pI is fairly homogeneous: a faint-staining, narrow "fringe" of protein surrounds the main pI, but extends only from pH 7.4 to 7.6, reflecting limited heterogeneity of charge. Considering the large overall mass of the protein and the relative paucity of bound carbohydrates, the sialylated glycan variants detected should not perturb the pI of DEC-205 excessively.

DEC-205 is very sensitive to proteolytic degradation. Precautions had to be taken to inhibit a broad range of proteases during the purification, and to remove the

cytosolic fraction after hypotonic lysis, or else the yield was very low. Proteolysis appears to proceed by a distinctly nonrandom pathway. Despite the continuous presence of high concentrations of six protease inhibitors in our buffers, we invariably observed a "ladder" of 6-8 discrete, minor, lower molecular mass bands, ranging down to about 80 kDa and containing the NLDC-145 epitope, whenever the antigen was blotted at high levels. This orderly array of proteolytic fragments could be observed in every preparation blotted, from crude thymic membrane extract (best seen in Figure 4, but also present in Figures 1B and 3B) to the purified, ultrafiltered immunoaffinity eluate (Figure 3B). The "fringe" of staining around the main isoelectric point is likely to be at least partly produced by these relatively large proteolytic fragments.

The large size, nonrandom distribution and relative protease resistance of the "ladder" of minor bands suggests to us that the complete primary structure of DEC-205 will reveal a modular architecture, with multiple protease-resistant domains joined by more protease-sensitive connecting segments.

EXAMPLE 2: DEC-205, A RECEPTOR EXPRESSED BY DENDRITIC CELLS AND THYMIC EPITHELIAL CELLS, HAS TEN C-TYPE LECTIN DOMAINS AND IS INVOLVED IN ANTIGEN PROCESSING

This example reports that DEC-205 is a receptor with ten C-type lectin domains which is homologous to the macrophage mannose receptor (MMR), and other related receptors that bind carbohydrates and mediate endocytosis. The function of DEC-205 was investigated with monoclonal and polyclonal anti-DEC-205 antibodies. It was determined that DEC-205 on dendritic cells is rapidly internalized via coated vesicles, and delivered to a multivesicular endosomal compartment that resembles the MHC class-II containing vesicles implicated in antigen processing. Furthermore, rabbit anti-DEC-205 antibodies were efficiently processed by dendritic cells and presented to rabbit IgG specific T cell clones. These experiments suggest that DEC-205 is a novel endocytic receptor that can be

used by dendritic cells and thymic epithelial cells to direct captured antigens from the extracellular space to a processing compartment.

Materials and Methods

5 *Purification of dendritic cells--* Dendritic cells from 7 day bone marrow cultures were treated with polyclonal rabbit anti-DEC-205 F(ab)'2 fragments and 10 nm gold-labeled goat anti-rabbit IgG as described in Figure 11 and processed for electron microscopy. For each time point 10 grids were examined, and all cells that were labeled with gold were photographed. gold particles were counted and
10 scored by a blinded observer based, on standard morphological criteria. The numbers in parentheses represent the percentage of total gold particles scored in each compartment.

Northern blotting-- For Northern blots, 2 µg of mRNA were electrophoresed in
15 0.8% agarose formaldehyde gels. Samples were transferred to nylon membranes and probed with an anti-sense RNA probe that spanned nucleotide positions 3688-5200 in the DEC-205 cDNA. The blot was subsequently stripped and re-hybridized with glyceraldehyde-3-phosphate dehydrogenase probe as a loading control.

20 *Electron microscopy--* Dendritic cells harvested from 7 day mouse bone marrow cultures were incubated with 10 µg/ml of either: polyclonal anti-DEC-205, Fab'2 fragments of polyclonal anti-DEC-205; or biotinylated monoclonal NLDC-145, on ice for 30'. Excess primary antibody was removed by washing cells 3 times with
25 RPMI-1640, 10% FCS, 0.02% NaN3. The cells were then incubated for 30' on ice with either: a 1:5 dilution of 10 nm gold labeled goat anti-rabbit IgG; or a 1:5 dilution of 10 nm gold labeled streptavidin respectively. Excess secondary reagent was removed by washing cells as above. Dendritic cells were then either fixed with 2.5% glutaraldehyde for a time-zero point, or incubated for the stated times at
30 37°C before fixation and processing for electron microscopy.

- Antigen presentaion--* 105 BALB/c mouse Dendritic cells obtained from day 7 bone marrow cultures were co-cultured with 105 2R.50 rabbit IgG specific T hybridoma cells for 48 hours in triplicate (Boom et al., J Exp Med 1988 167:1350-64). The supernatants were assayed for IL-2 concentration using the HT-2
- 5 indicator cell line. IL-2 production by the 2R.50 cells is plotted against the concentration of antibody in the cultures on a log scale. The error bars indicate the standard deviation from the mean.

Results and Discussion

- 10 This Example reports the molecular characterization of the 205 kDa cell surface protein described in Example 1. Using oligonucleotide probes based on the protein sequence, fourteen cDNA clones were obtained from three separate thymic and dendritic cell cDNA libraries. All of the cDNA clones were derived from the same mRNA. Clones containing the putative 5' end of the DEC-205 cDNA encoded the
- 15 N-terminal peptide of the DEC-205 antigen, and this was preceded by a hydrophobic leader consistent with a signal sequence. The protein contains ten C-type lectin domains, a transmembrane domain, and a cytoplasmic domain (Figure 7).
- 20 The composite cDNA had a single 5.2 Kb open reading frame encoding a protein of 1,722 a.a. with a predicted molecular weight of 195 Kda that included all 29 unambiguous DEC-205 peptide sequences (Figure 8). Of particular note is the high degree of sequence identity and similarity of the cytoplasmic domains of both murine and human DEC (Figure 9). A 7.5 Kb mRNA that corresponds to this
- 25 cDNA was expressed at high levels in dendritic cells, thymus, and lymph nodes, a pattern that corresponds to that which was obtained by staining tissues with the NLDC-145 monoclonal antibody (Kraal et al., *supra*) (Figure 10).

- The sequence of DEC-205 was aligned with known proteins in the database, and it
- 30 was determined that it is homologous to the macrophage mannose receptor (MMR) (Taylor et al., 1990, J. Biol. Chem. 265:12156-62; Ezekowitz et al., 1990, J. Exp.

Med. 172:1785-94) and the phospholipase A2 (PLA2) receptors of rabbit skeletal muscle (Lambeau et al., 1994, J. Biol. Chem. 269:1575-8), and to bovine pancreas (Ishizaki et al., 1994, J Biol Chem. 269:5897-904) (Figure 8). All known members of this family, which has been designated as the group VI C-type animal lectins

5 (Drickamer and Taylor, 1993, Annu. Rev. Cell Biol. 9:237-64), are type 1 transmembrane proteins with short cytoplasmic domains that mediate receptor endocytosis (Ezekowitz et al., 1990, J. Exp. Med. 172:1785-94; Ishizaki et al., 1994, J. Biol. Chem. 269:5897-904; Taylor et al., 1992, J. Biol. Chem. 267:1719-26). The extracellular portions of MMR family proteins have a distinctive

10 cysteine-rich N terminal domain, followed by a fibronectin type II repeat, and eight Ca^{++} dependent carbohydrate recognition domains (C-type CRDs) (Taylor et al., 1990, J. Biol. Chem. 265:12156-62; Lambeau et al., 1994 J. Biol. Chem. 269:1575-8; Ishizaki et al., 1994, J. Biol. Chem. 269:5897-904). DEC-205 diverges from this pattern only in that it has ten instead of the usual eight C-type CRDs (Figure

15 7). The functions of the cysteine-rich domain and the fibronectin repeat in group VI lectins have not been defined. By contrast, there is extensive experimental evidence that the C-type CRDs are carbohydrate-binding domains (reviewed by (Drickamer and Taylor, 1993, Annu. Rev. Cell Biol. 9:237-64)) and both the MMR and rabbit PLA2 receptor bind carbohydrates (Lambeau et al., 1994, J. Biol. Chem.

20 269:1575-8; Drickamer and Taylor, 1993, Annu. Rev. Cell Biol. 9:237-64). The carbohydrate contact residues defined for the C-type CRDs in rat serum mannose-protein and E-selection (Drickamer, 1992, Nature 360:183-86; Weis et al., 1991, Science 254:1608-15; Weis et al., 1992, Nature 360:127-34; Graves et al., 1994, Nature 367:532-8) are not conserved in DEC-205. However, additional

25 mechanisms must exist for carbohydrate binding by C-type CRDs since sequence features initially defined as "essential" for carbohydrate binding are also absent from the CRDs of NKR1, and rabbit PLA2 receptors which bind avidly to carbohydrate ligands (Lambeau et al., 1994, J. Biol. Chem. 269:1575-8; Bezouska et al., 1994, Nature 372:150-57). CRDs are found in over one hundred other

30 proteins (Drickamer and Taylor, 1993, Annu. Rev. Cell Biol. 9:237-64), but the CRDs in DEC-205 are most closely related to those found in the bovine and rabbit

PLA2 receptors, and the MMR (34.6% identity with bPLA2 receptor and 26.7% identity with hMMR). Indeed there is an ordered correspondence between CRDs 1-5 and 7-8 in DEC-205 and CRDs 1-5 and 7-8 in other group VI animal lectins, whereas CRD 6 of DEC-205 most closely resembles the first CRDs in other family members. The unusual CRDs in DEC-205, numbers 9 and 10, are most closely related to CRDs 7 and 8 in other group VI lectins, and may have arisen during a gene duplication event. At least two of the CRDs in the MMR are known to bind mannose (Taylor et al., 1992, J. Biol. Chem. 267:1719-26), and grouping of several CRDs increases the affinity of the MMR for carbohydrate ligands (Taylor and Drickamer, 1993, J. Biol. Chem. 268:399-404. The same mechanism may be utilized by DEC-205 to increase both the affinity and diversity of carbohydrates bound by this receptor.

The receptor's function was investigated using a combination of monoclonal and polyclonal anti-DEC-205 antibodies. The observation that the cytosolic domain of DEC-205 contains conserved aromatic amino acids (Figure 9; SEQ ID NOS:1 and 6), which have been implicated as part of an endocytic motif (Ezekowitz et al., 1990, J. Exp. Med. 172:1785-94; Ishizaki et al., 1994, J. Biol. Chem. 269:5897-904; Chen et al., 1990, J. Biol. Chem. 265:3116-3123; Collawn et al., 1990, Cell 63:1061-72), suggested that this receptor might be used by dendritic cells and thymic epithelial cells to deliver a variety of extracellular glycoprotein antigens to an intracellular antigen processing compartment. To test this idea, the fate of immunogold-labeled monoclonal and polyclonal anti-DEC-205 antibodies bound to DEC-205 on dendritic cells was examined by electron microscopy in a time course experiment (Figure 11). Similar results were obtained using the monoclonal antibody and intact or F(ab')₂ fragments of polyclonal antibodies in two separate experiments. At time zero, 95% of the cell-associated gold particles were found on the plasma membrane. After warming the sample to 37°C for only one minute, 38% of the particles were found in coated vesicles or coated pits. By 20 minutes after crosslinking, 79% of the gold particles were in a multivesicular compartment that is characteristic of dendritic cells (Steinman et al., 1979 J. Exp. Med. 149:1-

16; Kleijmeer et al., 1994, J. Invest. Dermatol. 103:516-523) and resembles the MHC class-II containing vesicles that are thought to be involved in antigen processing (Amigorena et al., 1994, Nature 369:113-120; Qiu et al., 1994, J. Cell Biol. 125:595-609; West et al., 1994, Nature 369: 147-151; Tulp et al., 1994, Nature 369:120-26; Schmid and Jackson, 1994, Nature 369:103-4) (Figure 11 and Table 2). Thus, DEC-205 is rapidly internalized via coated vesicles, and antibodies bound to the internalized receptor are delivered to multivesicular endosomal compartment.

TABLE 2

Compartmental localization of gold particles by electron microscopy

	<u>Plasma</u> <u>Membrane</u>	<u>Coated</u> <u>Pit/Vesicle</u>	<u>Multi-vesicular</u> <u>Endosome</u>	
<u>Lysosome</u>				
0'	156 (95%)	8 (5%)	0	0
1'	68 (62%)	31 (38%)	0	0
5'	129 (41%)	83 (28%)	99 (32%)	0
20'	12 (5%)	3 (1%)	175 (79%)	32
(14%)				
60'	17 (7%)	9 (5%)	46 (19%)	172
(70%)				

To determine whether DEC-205 could deliver antigen to an active antigen processing compartment, dendritic cells were treated with rabbit anti-DEC-205 antibodies, and assayed for presentation of rabbit IgG-peptide/MHC complexes to T cell clones (Boom et al., 1988, J. Exp. Med. 167:1350-64). Negative controls included non-specific rabbit antibodies, and rabbit antibodies to IgG2a that are efficiently presented by B cell lines (Boom et al., 1988, J. Exp. Med. 167:1350-64). It was determined that dendritic cells presented rabbit anti-DEC-205 to the T cells clones two orders of magnitude more efficiently than the non-specific rabbit antibodies or rabbit anti-IgG2a (Figure 12). Thus, DEC-205 resembles membrane immunoglobulin on B cells in that the crosslinked receptor is efficiently

internalized and bound ligands are delivered to an intracellular compartments that are active in antigen processing (Chestnut and Gray, 1981, J. Immunol. 126:1075-79; Rock et al., 1984, J. Exp. Med. 160:1102-25; Lanzavecchia, 1985, Nature 314:538-39).

5

In conclusion, dendritic cells and thymic epithelial cells express a novel receptor, DEC-205, which contributes to antigen presentation. The multi-lectin domain structure suggests that this receptor can be used by dendritic cells and thymic epithelial cells to capture and endocytose diverse carbohydrate bearing antigens and

10 direct them to an antigen processing compartment.

EXAMPLE 3: EXPRESSION OF THE DEC-205 ON DENDRITIC CELLS
AND OTHER SUBSETS OF MOUSE LEUKOCYTES

15

Prior studies by a variety of groups demonstrated that the mAb NLDC-145 reacted primarily with dendritic cells (DCs) and the epithelial cells of the thymic cortex. As shown in Example 1, this mAb recognizes DEC-205, a 205 kDa integral membrane glycoprotein with a unique amino-terminal sequence, and a rabbit

20 polyclonal antibody to purified DEC-205 with higher affinity for the blotted antigen than the original mAb was generated. Both the polyclonal and NLDC-145 antibodies have been used in this Example to reassess the expression and function of DEC-205 on leukocytes. Cytofluorography revealed that DCs derived from the epidermis (Langerhans cells) and from proliferating bone marrow progenitors

25 (BMDCs) expressed high levels (2-3 logs) of DEC-205, while freshly-isolated spleen DCs comprised two subsets, most (80%) staining at low levels (≤ 1 log), the remainder moderately (1.5 logs). DEC-205 epitopes were sensitive to trypsin, but were regenerated in culture. Resident and inflammatory peritoneal macrophages did not express the antigen, except for small amounts on thioglycollate-elicited

30 cells. B cells from spleen, lymph node, bone marrow, blood and peritoneal fluid expressed levels of DEC-205 that were 10 to 50-fold lower than those on BMDCs. Marrow pro- and pre-B cells did not express DEC-205. Polyclonal anti-DEC-205 failed to inhibit either stimulation of a primary mixed leukocyte reaction by DCs *in*

vitro, or a local graft vs. host response *in vivo*, where parental T cells were injected into F1 mice. DEC-205 is therefore more broadly expressed on leukocytes than previously appreciated.

- 5 The monoclonal antibody described in Kraal et al. (1986, J. Exp. Med. 163:981) was not able to block any DC functions tested, either *in vitro* or in mice given long-term injections of NLDC-145 IgG from birth (Breel et al., 1988, Immunol. 63:331). Because of the unique tissue distribution of the antigen recognized by NLDC-145, and because of its abundant expression on a cell type (dendritic cells)
- 10 for which few restricted mAbs have been identified, the cell specificity and potential function of DEC-205 was reexamined. The new polyclonal antibody (described in Example 1) was used to improve detection of DEC-205, and to attempt to perturb DC function.

15 Materials and Methods

Mice-- Adult (6 to 10 wk old) mice of both sexes were studied, including (C57BL/6 x DBA/2) and (BALB/C x DBA/2) F1 mice from the Trudeau Institute (Saranac Lake, NY), and (C57BL/6 x BALB/C) F1 and BALB/C mice from Japan SLC (Hamamatsu, Shizuoka, Japan).

- 20 *Cell Suspensions*-- Cells were studied either immediately after isolation from the animal or following a period of culture in RPMI-1640 medium supplemented with 5% FCS, 50 μ M 2- mercaptoethanol, and 20 μ g/ml gentamicin. Spleens, thymi, and lymph nodes were either teased with forceps, or additionally digested with
- 25 collagenase (Swiggard et al., 1992, *In Current Protocols in Immunology*. Coligan et al., (Eds). Green Publishing Associates and Wile Interscience: New York Supplement 3, pp. 3.7 1-11; Crowley et al., 1989, Cell Immunol. 118:108), with similar results. Bone marrow cells were flushed with a syringe from femurs and tibias, while blood was obtained by cardiac puncture in heparin. All cell
- 30 suspensions were depleted of red cells by lysis in 0.83% ammonium chloride solution. Dendritic cells were obtained from three sources, each as described: the

epidermal sheets of mouse ears (Schuler and Steinman, 1985, J. Exp. Med. 161:526), the low density plastic adherent population of spleen (Saveggard et al., *supra*; Crowley et al., *supra*), and proliferating bone marrow progenitors that were expanded in rGM-CSF (Inaba et al., 1992, J. Exp. Med. 176:1693). Peritoneal

5 cells were either resident populations or were elicited by various inflammatory stimuli: proteose peptone 3 days earlier, thioglycollate broth 4 days earlier, 50 μ g concanavalin A 2 days earlier, or 10^7 live *Mycobacterium bovis* BCG organisms 7 days earlier. Several populations were also studied after a period of 1-3 days in culture. The B cells in lymph node suspensions were stimulated with the B cell

10 mitogens lipopolysaccharide (10 μ g/ml LPS, from *E. Coli* 0111:B4, Difco, Detroit, MI), anti-IgM plus IL-4 (10 μ g/ml goat F(ab')₂ anti-mouse IgM, Jackson ImmunoResearch (West Grove, PA), plus 50 U/ml of recombinant murine IL-4, kind gift of Dr. T. Sudo, Basic Research Laboratories, Toray Industries, Kamakura, Japan), and CD40 ligand (CD40L: L cells transfected with CD40L (kind gift of

15 Dr. H. Yagita, Juntendo University School of Medicine, Japan), fixed with 1% paraformaldehyde and washed 3 times in PBS before 1:1 coculture with B cells). Dendritic cells in skin were cultured as whole epidermal suspensions and then enriched by flotation on dense bovine albumin (Schuler and Steinman, *supra*), while dendritic cells in spleen were cultured from low density spleen adherent cells,

20 with or without supplementation in rGM-CSF (200 U/ml) or keratinocyte-conditioned medium, as described (Witner-Pack et al., 1987, J. Exp. Med. 166:1484).

Two-color labeling methods were used to simultaneously identify a particular

25 subset of leukocytes (PE-labeled antibody) and DEC-205 (NLDC-145 rat mAb or rabbit polyclonal anti-DEC-205 followed by FITC labeled anti-Ig). Nonreactive control antibodies were nonimmune rat IgG2a (Zymed, South San Francisco, CA) and rabbit IgG (Jackson ImmunoResearch; intact IgG or F(ab')₂ prepared by us). The staining sequence was: (a) primary anti-DEC-205 or nonimmune; (b)

30 secondary anti-Ig (FITC conjugates of mouse anti-rat IgG or goat anti-rabbit F(ab')₂, both from Jackson ImmunoResearch); (c) rat or rabbit IgG at 10 μ g/ml to

quench; and (d) the PE- or biotin-labeled antibody. The latter reagents were purchased from PharMingen (La Jolla, CA), and were: biotin conjugates directed to class II MHC (clone AMS-32.1), B220/CD45RB (clone RA3-6B2) and Thy-1.2/CD90 (clone 53-2.1) antigens; or PE conjugates directed to Mac-1/CD11b (clone M1/70) and Gr-1 granulocyte (clone RB6-8C5) antigens. At least 10,000 cells per sample were examined in a FACScan cytofluorograph (Becton Dickinson Immunocytometry Systems, Mountainview CA).

Immunoblotting-- This was performed with monoclonal and polyclonal reagents, as described (in Example 1, *supra*).

Immunocytochemistry-- Cytospins were fixed in neat acetone for 10 min at room temperature, air-dried, and stained with antibodies exactly as described for cytofluorography. The same secondary reagents were used, except that peroxidase conjugates instead of FITC conjugates were employed. Staining was visualized with diaminobenzidine (Stable DAB, Research Genetics, Huntsville, AL).

Functional Studies-- Monoclonal (10 and 1 $\mu\text{g/ml}$) and polyclonal (30 and 10 $\mu\text{g/ml}$) antibodies were applied at doses that were close to or above saturation, continuously, to a one-way allogeneic mixed leukocyte reaction (MLR), wherein 3×10^5 nylon wool-passed lymph node T cells were stimulated by graded doses of allogeneic irradiated or mitomycin-treated DCs (Inaba and Steinman, 1984, J. Exp. Med. 160:1717; Inaba et al., 1987, J. Exp. Med. 166:182). Syngeneic MLRs were run in tandem. The positive control for MLR inhibition utilized a reagent that interferes with the B7 costimulation system (GL-1 rat mAb to B7-2) (Hathcock et al., 1993, Science 262:905). For *in vivo* experiments, we used the local graft-versus-host (GVH) reaction (Atkins and Ford, 1975, J. Exp. Med. 141:664), wherein parental lymph node cells injected into the hind foot pad induce a GVH in the draining popliteal node of F1 mice, presumably upon encountering F1 dendritic cells in the lymph node cortex. Lymph node weights of control (PBS-injected) and GVH nodes were measured at day 7, using 5 mice per group. The protocol was to inject 200 μg of anti-DEC-205 or control IgG into the foot pad at time 0, and to

repeat the Ig injection 8 hours later, adding either 10^7 parental lymph node cells or a corresponding volume of PBS to the injected solution.

Results

5 *Expression of DEC-205 by epidermal dendritic cells.* Three forms of antibody to DEC-205 (monoclonal NLDC-145, polyclonal anti-DEC-205 IgG, and polyclonal anti-DEC-205 F(ab')₂ fragments) were applied to cultured epidermal cells. Prior data had shown strong staining of Langerhans cells by NLDC-145. Since the NLDC-145 epitope is trypsin-sensitive (below), and since trypsin is used initially to
10 prepare the sheets of epidermis from which Langerhans cells are released, we concentrated on epidermal cells that had been cultured overnight, to allow time for the protein to be repleted on cell surfaces. The culture period also provides time for most keratinocytes to adhere to the plastic surface, and for the nonadherent Langerhans cells to acquire a low buoyant density (Crowley et al. *supra*; Schuler
15 and Steinman, *supra*). As a result, preparations with 20-50% dendritic cells can be obtained by studying nonadherent, overnight cultures of epidermal cells, especially following flotation on columns of dense BSA.

Epidermal cells were stained with a phycoerythrin (PE)-tagged mAb to class II
20 MHC proteins, to distinguish Langerhans cells from keratinocytes, and were counterstained with hybridoma supernatants of NLDC-145 and mAbs to other leukocyte lineages (Figure 13, A-D). The specificity of NLDC-145 for dendritic cells (Figure 13A) was demonstrated by the fact that isotype-matched IgG2a mAbs to macrophages (SER-4 anti-sialoadhesin (Coocker and Gordon, 1989, J. Exp. Med.
25 169:1333)), B cells (RA3-6B2 anti-B220 (Hoffman and Weissman, 1981, Nature 289:681)), and T cells (53-6.72 anti-CD8; (Ledbetter and Herzenberg, 1979, Immunol. Rev. 47:63)) were non-reactive with NLDC-145 (+) cells (Figure 13, panels B-D). When the same suspensions were counterstained with graded doses of either purified NLDC-145 IgG (rat IgG2a κ) or polyclonal, nonimmune rat IgG2a as
30 a nonreactive control, staining of Langerhans cells by NLDC-145 reached a plateau

at 2 $\mu\text{g/ml}$ (Figure 13, compare the staining of the arrowed MHC-II (+) dendritic cells in panels E-G with H).

The anti-DEC-205 rabbit polyclonal was also applied, both as F(ab')_2 fragments and as intact IgG, and was compared with nonimmune F(ab')_2 and IgG over a broad range of doses (0.3-100 $\mu\text{g/ml}$). The rabbit reagents did react with the class II MHC-negative keratinocytes, but this binding was entirely nonspecific, since the staining was comparable with immune and nonimmune reagents (Figure 13, compare panels I-L with M-P, and Q-T with U-X). The staining of MHC-II (+) Langerhans cells, however, was strong, specific, and comparable using anti-DEC-205 as either intact IgG or F(ab')_2 fragments, with apparent saturation at 30 $\mu\text{g/ml}$ (Figure 13, panels I-L for F(ab')_2 fragments, panels Q-T for intact IgG).

The second rabbit polyclonal antibody, raised to a synthetic peptide spanning the first 19 residues of DEC-205, failed to stain Langerhans cells, instead giving a pattern like that of nonimmune IgG (not shown).

The trypsin sensitivity of DEC-205 epitopes was examined. Partially-enriched cultured Langerhans cells were either not treated or were exposed to trypsin (0.25% in PBS for 30 min on ice). Staining by both monoclonal and polyclonal reagents was decreased by ten-fold (1 log of fluorescence intensity in Figure 14 compare A and B to C and D). The epitopes were reexpressed, to levels equal to those on untreated cells, during a subsequent overnight culture period (Figure 14, compare E and F with A and B).

25

Expression of DEC-205 by other dendritic cell populations. Spleen DCs were identified in low buoyant density splenocyte suspensions (See Example 1; and Surggord et al., *supra*) by their expression of the integrin CD11c (Figure 15A, C, E and G), as detected with the mAb N418 (Metlay et al., 1990, J. Exp. Med. 171:1753). Counterstaining with either NLDC-145 or with the anti-DEC-205 polyclonal revealed that, whether freshly isolated or cultured overnight, these cells

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expressed less DEC-205 on their surfaces than Langerhans cells (Figure 15C, D, G and H). Freshly-isolated spleen DCs comprised two phenotypic subsets, as previously described (Crowley et al., *supra*). Most (roughly 80%) expressed relatively low but detectable levels (≤ 1 log) of the antigen, while a smaller
5 population stained moderately (ca. 1.5 logs: *arrows*, Figure 15C, D, G, and H). After overnight culture, DEC-205 expression by all of the CD11c (+) DCs had risen to the moderate (1.5 log) level, but never to the levels observed on epidermal dendritic cells (ca. 2 logs: *arrows*, Figure 15K, L, O and P) attempts to further upregulate expression of DEC-205 by culturing the cells in rGM-CSF, or in
10 keratinocyte-conditioned medium containing GM-CSF, did not yield an increase beyond that induced by culture alone (not shown).

In contrast, when dendritic cells were generated from bone marrow progenitors with GM-CSF (Inaba et al., 1972, *supra*), their expression of DEC-205 was
15 uniformly high, comparable to the levels on Langerhans cells (not shown). Interestingly, the actively proliferating populations at day 6 of the marrow cultures contained relatively few cells that expressed DEC-205 (Figure 16), but expression had increased to uniformly high levels by day 8.

20 *Expression by resident and elicited peritoneal cells.* Resident peritoneal cells (consisting of about 30% macrophages and 70% B cells) were compared to inflammatory peritoneal cells in exudates elicited with concanavalin A (Con A), thioglycollate (TGC), or live *M. bovis* Bacille Calmette-Guérin (BCG) organisms (Figure 17). The data are shown for polyclonal nonimmune and immune F(ab')₂
25 fragments. Similar staining was obtained with the NLDC-145 monoclonal (not shown). However, nonimmune and immune intact rabbit IgG gave strong background staining on peritoneal macrophages, presumably because of binding to Fc receptors (not shown). Resident, Mac-1 (+) peritoneal macrophages did not express surface DEC-205, but peritoneal B cells expressed measurable levels
30 (roughly 1 log above background: Figure 17 E-H *arrows*). Macrophages in Con A- and BCG-elicited exudates again showed little or no staining with anti-DEC-205

(Figure 17 E-H, *arrowheads*), even though these macrophages were all strongly class II MHC-positive (not shown). The Con A and BCG exudates contained significant numbers of T cells, but these did not stain with anti-DEC-205 (anti-Thy-1 double label not shown). In contrast to the other peritoneal populations tested, 5 TGC-elicited macrophages did express DEC-205, albeit at low levels (0.5-1 log above background). In each of the resident and elicited populations, B cells stained comparably.

Expression of DEC-205 by resident leukocytes in multiple tissues, particularly B
10 *cells.* Given the surprising finding that resident peritoneal B cells expressed DEC-205, we explored the distribution of the antigen further by examining cell suspensions from spleen, bone marrow, peripheral blood, lymph node, and thymus for co-expression of DEC-205 with several leukocyte markers. Results with the first 3 organs are illustrated here (Figure 18). The results with spleen and lymph 15 node were identical (not shown). Thymocytes stained only marginally (<0.5 log above background (not shown).

In spleen suspensions, B cells (B220 and MHC-II (+); Gr-1, Mac-1 and Thy-1 (-): Figure 18 F-J, *arrows*) again expressed DEC-205, staining approximately one log 20 above background. T cells (Thy-1 (+)) and granulocytes (Gr-1 (+), Mac-1 (+)) also stained, but less strongly than B cells.

In bone marrow (Figure 18, K-T), granulocytes stained almost a log above background, whereas marrow B cells displayed heterogeneous levels of DEC-205. 25 Most B220 (+) cells in the marrow stained weakly or not at all, while a subset of cells with higher levels of B220 (*arrow*, Figure 18, P-T, B220 double label) co-expressed DEC-205 at levels comparable to those on peripheral B cells. These B220^{bright}.DEC-205 (+) cells also expressed surface IgM, identifying them as mature B cells (not shown). A small subset of Mac-1 (+), B220 (-) cells -- presumably 30 monocytes -- did not express DEC-205.

In peripheral blood (Figure 18 U-δ), B cells (*arrows*) stained comparably to B cells in lymphoid tissues, while granulocytes and T cells showed weaker but measurable staining.

- 5 To gain knowledge of the relative amounts of DEC-205 expressed by different cell types (Figure 19), graded doses of whole-cell NP-40 lysates from bone marrow dendritic cells, splenocytes (about 65% B cells) and peritoneal cells (about 70% B cells and 30% macrophages) were immunoblotted. The signal from 10,000 BMDCs was approximately twice as strong as the signal from 100,000 splenocytes,
10 corresponding to at least ten times more DEC-205 per cell in BMDCs than splenic B cells. The signal from 100,000 peritoneal cells was weaker still, 5-10 times fainter than the signal from 10,000 DCs, corresponding to about 50 times less DEC-205 in peritoneal B cells than BMDCs.
- 15 To examine the effects of B cell activators on surface levels of DEC-205, B cells were cultured for up to 2 days in the presence of LPS, CD40 ligand, and the combination of anti-IgM and IL-4. None induced a significant increase, but the latter combination induced a modest (2-fold) decrease in surface levels of DEC-205, as detected with both monoclonal and polyclonal reagents (not shown).
- 20
- Mouse B cell viability is greatly improved during stimulation in culture, allowing us to ask whether the DEC-205 epitopes on B cells were actively synthesized by the cells, rather than adsorbed on their surfaces from an extracellular source. On B cells, as on Langerhans cells, trypsin treatment eliminated most of the staining by
25 NLDC-145 and anti-DEC-205, but the epitopes were resynthesized during overnight culture (Figure 14 G-L).

Attempts to block dendritic cell function with antibodies to DEC-205. When one-way mixed leukocyte reactions were performed in the continuous presence of either
30 10 µg/ml of NLDC-145, or 30 µg/ml polyclonal anti-DEC-205, no inhibition of allogeneic T cell proliferation was observed (Figure 20). The rat IgG2a

monoclonal GL-1, directed against the costimulator protein B7-2 (Hathcock et al., *supra*; Freeman et al., 1993, J. Exp. Med. 178:2185; Inaba et al., 1994, J. Exp. Med. 180:1849), was utilized as a positive blocking control. GL-1 inhibited proliferation in this system, but did not abolish it, as expected: blockade of
5 multiple costimulators is required to completely ablate proliferation in an allogeneic MLR (Young et al., 1992, J. Clin. Invest. 90:229).

To ascertain whether DEC-205 might play a role in the homing of T cells to the T-dependent areas of lymphoid tissue, where dendritic cells express high levels of
10 DEC-205 (Kraal et al., *supra*; Example 4, *supra*), the local graft-versus-host (GVH) reaction was studied (Atkins and Ford, *supra*). This response is produced in the draining popliteal lymph node of F1 mice when parental-strain lymph node cells are injected into the hind foot pad. The T cells migrate to the draining node, where they encounter allogeneic MHC proteins, presumably initially on dendritic
15 cells and within the cortical T cell areas. A substantial dose of nonimmune or anti-DEC-205 F(ab')₂ fragments (200 µg) was injected into the footpad at 0 hours. Eight hours later, a second dose of antibody along with 10 million lymph node cells (ca. 70% T cells) was injected. The T cells induced a strong GVH reaction: the draining lymph nodes swelled to 5-6 times their normal size within 7 days.
20 Polyclonal anti-DEC-205 was unable to inhibit this primary T cell response *in vivo* (Table 3).

Table 3: Failure of polyclonal anti-DEC-205 to inhibit local GVH *in vivo*

F1 footpad	Weight of popliteal lymph node (g)		
	Mouse number	No cells injected	10 ⁷ parental lymph node cells injected
Nonimmune F(ab') ₂	1	2.63	13.27
	2	2.36	13.83
	3	2.89	12.01
	4	2.06	12.80
	5	2.14	13.93
10	Mean difference (GVH) = 10.75 ± 1.03 g		
Anti-DEC-205 F(ab') ₂	1	2.88	14.91
	2	2.08	12.01
	3	2.22	13.19
	4	1.95	11.31
	5	2.38	13.33
15	Mean difference (GVH) = 10.65 ± 1.03 g		

20

Discussion

Prior studies failed to detect the DEC-205 antigen on most types of leukocytes except for dendritic cells (Kraal et al., *supra*; Crowley et al., *supra*). This Example demonstrates that other leukocytes, especially B cells, can express DEC-205, although at much lower levels. Example 4 reports low-level expression of DEC-205 by B cells in tissue sections. Polyclonal rabbit antibodies raised against the N-terminal peptide of DEC-205 and the intact protein described in Example 1 were used. The anti-peptide reagent failed to stain live cells, suggesting that under native conditions, the amino terminus of DEC-205 may be involved in a higher-order protein structure that alters its conformation relative to the 19-residue synthetic immunogen, or sterically hinders access by an antibody. The failure of the anti-peptide polyclonal to bind cells cannot be explained by covalent modification of the N-terminal serine of DEC-205 in tissues, since this residue was susceptible to Edman degradation in protein isolated from thymi. In contrast, the

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polyclonal raised to intact DEC-205 stained cells with patterns of reactivity that mimicked NLDC-145 closely.

- Differences in DEC-205 expression among different classes of leukocytes were observed. Other than dendritic cells, B cells expressed the most DEC-205, although their levels were 10 to 50 times lower than those on BMDCs (Figure 19). The DEC-205 detected on B cell surfaces was actively synthesized by the cells themselves, and unlikely to be adsorbed to their surfaces from an extracellular source, since after trypsinization, DEC-205 epitopes were regenerated in culture.
- Expression of DEC-205 appears to be coordinated with the developmental transition from pre-B cell to surface IgM (+) B cell in the marrow. However, peripheral B cell stimulation with a variety of mitogens (LPS, CD40 ligand, anti-IgM plus IL-4) was not accompanied by a significant increase in surface expression of DEC-205. Granulocytes expressed DEC-205, with higher levels on granulocytes in bone marrow than in blood. Thymocytes and mature T cells from spleen and lymph node expressed very low but detectable levels of DEC-205, whereas T cells in blood and peritoneal fluid did not express detectable levels. It is possible that the DEC-205 detected on granulocytes and T cells was adsorbed from surrounding stromal cells that are rich in DEC-205, such as bone marrow stroma, thymic epithelium, and the dendritic cells in the T cell areas of peripheral lymphoid tissues. Most macrophage populations lack DEC-205, although thioglycollate-elicited cells are weakly positive, as previously described (Wiffels et al., 1991, Immunobiol. 184:83).
- Although other leukocyte lineages express DEC-205, it is evident that dendritic cells express some 10-50 times more of the antigen, as assessed by immunoblotting. DEC-205 expression is regulated on dendritic cells in some way. Freshly isolated splenic DCs have relatively little DEC-205, and the levels increase only modestly in culture. The dendritic cells that express high levels of DEC-205 are those in skin, in the T cell regions of peripheral lymphoid organs, and dendritic

cells that are grown from proliferating bone marrow precursors in the presence of high-dose GM-CSF.

To look for a contribution of DEC-205 to immune responses in tissue culture systems, we attempted to inhibit the primary allogeneic MLR. It was hypothesized that DEC-205 might be important in the capacity of dendritic cells to interact with helper T cells. However, either monoclonal nor polyclonal antibodies were able to block this T cell response with either monoclonal or polyclonal antibodies. We also attempted to inhibit an *in vivo* response, the local GVH reaction, in which parental T cells injected into nonlymphoid tissue migrate to the lymph node that drains the injection site and initiate an alloreactive response against F1 dendritic cells and B cells. Again, antibodies to DEC-205 had no effect. These negative results could have trivial explanations, such as clearance of the antibody, or compensatory resynthesis of new DEC-205 protein. Given the large molecular mass of DEC-205, it is possible that both monoclonal and polyclonal antibodies bind epitopes on the native protein that do not interfere with its functions in the responses studied. Alternatively, the assay systems may be those in which DEC-205 plays no critical role. DEC-205's putative function in immune responses appears to involve earlier events than those studied here, such as the acquisition of antigens by accessory cells, or selection events in lymphocyte development. Further inquiries into the function of DEC-205 are enabled by the molecular cloning of the antigen described in Example 2, *supra*.

EXAMPLE 4: EXPRESSION OF THE DEC-205 PROTEIN *IN SITU* IN LYMPHOID AND NONLYMPHOID TISSUES

In this Example, the monoclonal and polyclonal antibodies to DEC-205 were used to reassess the tissue distribution of DEC-205 by immunohistochemical staining of frozen sections from a variety of organs, and by multiple-organ immunoblotting.

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In an effort to better define the tissue distribution of DEC-205, we have examined a variety of tissues histologically and on immunoblots, using both monoclonal and

polyclonal antibodies, along with secondary anti-Ig reagents with greater sensitivity than those used in prior studies (Kraal et al., 1986, J. Exp. Med. 163:981; Crowley et al., 1989, Cell. Immunol. 118:108; Vremec et al., 1992, J. Exp. Med. 176:47; Austyn et al., 1994, J. Immunol. 152:2401; Lu et al., 1994, J. Exp. Med. 179:1823; Breel et al., 1988, Immunology 63:657). Abundant expression of DEC-205 was confirmed histologically on thymic and intestinal epithelia and on dendritic cells in the T cell areas of peripheral lymphoid organs. In addition, DEC-205 was visualized in several other locations: B lymphocytes within B cell follicles, the stroma of the bone marrow, the epithelia of pulmonary airways, and the capillaries of the brain. Immunoblotting confirmed the presence of substantial levels of DEC-205 protein in lysates prepared from lymphoid tissues and from lung, marrow and intestine. Thus, while DEC-205 is expressed at high levels by dendritic cells, it is also expressed by a number of other cell types *in situ*.

15

Material and Methods

Mice -- Adult (6-12 week old) female mice of three strains were studied: inbred C57BL/6 x DBA/2 (Trudeau Institute, Saranac Lake, NY) and BALB/C, and outbred CD-1 Swiss-Webster (the latter 2 strains from Taconic Farms, Germantown, NY).

20

Immunohistology -- Immediately after organs were removed, they were frozen at -20°C in O.C.T. tissue embedding medium (Miles, Elkhart, IN), and stored at -20°C. Tissue sections, usually 10 µm thick, were cut on a Minotome cryostat (IEC division of Damon, Needham Heights, MA) and applied to 10-well slides (Carlson Scientific, Peotone, IL). The sections were fixed in neat acetone for 10 min at room temperature, and air-dried. Subsequent steps were performed in a humid chamber. Sections were rehydrated in a drop (30-50 µL) of PBS, then primary antibody was applied. Hybridoma supernatants were used either undiluted or diluted 1:5 in PBS + 1% BSA, depending on their titer. Purified IgGs, ascites fluids and antisera were diluted in the same medium to optimized doses determined by titration, usually 1-10 µg/ml for purified protein and 1:3000-1:1000 for ascites

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and hyperimmune sera. The primary antibodies were the NLDC-145 mAb, applied either as a hybridoma culture supernatant or as purified IgG (protein G eluate), or rabbit polyclonal anti-DEC-205, (Example 1 *supra*), applied either as intact IgG (protein A eluate) or as F(ab')₂ fragments. Positive controls included rat mAbs to other subsets of leukocytes (RA3-6B2 anti-B220/CD45RB (TIB 146, ATCC, Rockville, MD), SER-4 anti-macrophage, and 53-6.72 anti-CD8 (TIB 105, ATCC)) and MHC class II proteins (M5/114 (TIB 120, ATCC)), as well as a rabbit polyclonal to Ig β , one of the signaling chains that associates with B cell surface Ig (Sanchez et al., 1993, J. Exp. Med. 178:1049). Negative controls were polyclonal rat IgG2a (Zymed, South San Francisco, CA) and rabbit IgG (Jackson ImmunoResearch, West Grove, PA), either intact or as F(ab')₂ fragments. Primary antibodies were left in contact with the sections for 45 min at room temperature, then the sections were washed 5 times with PBS, never allowing the sections to stand dry for more than a few seconds. Anti-Ig secondary antibodies were added next, usually donkey F(ab')₂ - horseradish peroxidase conjugates (Jackson ImmunoResearch), diluted 1:300 in PBS + 1% BSA. After a 45 minute incubation, sections were washed 5 times with PBS. The chromogenic substrate was a ready-to-use formulation of diaminobenzidine containing H₂O₂ (Stable DAB, Research Genetics, Huntsville, AL). Sections were washed 5 times in PBS. and usually were counterstained with Gill's Hematoxylin #1 (Fisher, Fair Lawn, NJ). Coverslips were attached using Permount histological mounting medium (Fisher).

Extraction and immunoblotting of protein from multiple organs -- Organs were placed in 10 volumes of a monophasic solution of phenol and guanidinium isothiocyanate (TRIzol, Gibco-BRL, Gaithersburg, MD) (Chomczynski, 1993, BioTechniques 15:532). Organs were homogenized for 15-30 s (Polytron, Brinkmann, Westbury, NY). RNA and DNA were removed by CHCl₃ extraction and ethanol precipitation. Proteins were precipitated from the phenol-ethanol supernatants with isopropanol (150% of the original TRIzol volume). and were redissolved in 1% SDS (30% of the original TRIzol volume, 50°C, 1 h). Extracts were clarified (3000 x g, 10 min, room temperature), and total protein levels were

measured (BCA assay, Pierce, Rockford, IL). Immunoblotting was performed as described (Chomczynski, *supra*), normalizing protein loads to 50 μ g per lane. Filters were stained with either 10 μ g/ml of NLDC-145 IgG or mAb 1D4B (anti-LAMP-1) hybridoma supernatant, diluted 1:1.

5

Results

In every organ studied, similar results were obtained with the NLDC-145 monoclonal antibody and with the rabbit polyclonal antibody raised to purified DEC-205 protein. This will be illustrated in several lymphoid and nonlymphoid
10 tissues. In many instances, DEC-205 was found to be expressed in sites that had not been described in prior work.

Thymus. Staining patterns in this organ were identical to those in the original description of the NLDC-145 mAb (Kraal et al., *supra*). Very strong peroxidase
15 immunolabeling was observed on thymic cortical epithelium, while weaker staining was noted on scattered dendritic profiles in the medulla (M, Figure 21, panels *a-c* and *g*).

Lymph node. Strong DEC-205 expression was apparent on dendritic profiles
20 throughout the T cell regions of the cortex (T, Figure 21 *d-f*). At higher power, there was a punctate character to the staining in the T cell areas of the deep cortex (Figure 21 *h*). The punctate pattern could represent the presence of DEC-205 in intracellular granules of dendritic cells and/or DEC-205 on the surfaces of many fine processes. No staining was evident in the medulla (M, Figure 21 *d-f*). Weak
25 staining for DEC-205 was evident on B cells in the follicles (B, Figure 21 *d-f*). This staining was obscured when hematoxylin was used to counterstain nuclei (Figure 21 *d-f*), but was clearly visible when hematoxylin was omitted (Figure 21 *i*).

30 *Spleen.* Strong staining for DEC-205 was evident in the T cell areas, *i.e.*, the periarterial sheaths (Figure 22: the central artery of the T area is arrowed in each

micrograph). The extent of staining for DEC-205 in the T areas paralleled that seen with anti-MHC class II (Fig. 21, compare *b* with *c*, and *d* and *e* with *f*), but the DEC-205 stain was again punctate in nature (Figure 22 *d-e*), as it was in the T cell areas of lymph nodes (above). As in lymph nodes (above), the B cell follicles (B, Figure 22 *a-c*) stained for DEC-205, although this staining was too weak to be evident in a black and white micrograph of a specimen counterstained with hematoxylin (Figure 22, compare the strong B cell staining with anti-Ig β in panel *a* and anti-MHC-II in panel *c* with the anti-DEC-205 staining in panel *b*). Anti-DEC-205 did not stain the marginal zone (see *arrowheads* to the marginal sinus, which stands out in panels *a* and *c* because of the strong B cell staining).

Brain. Both NLDC-145 and polyclonal anti-DEC-205 reagents produced linear staining along capillaries (*arrows*, Figure 23 *a-c*) and small arteries (*arrow*, Figure 23 *d*) in the cerebrum and cerebellum. Staining of capillaries was not observed in any other organ studied.

Lung. Many strongly stained DEC-205 profiles were scattered about the lung parenchyma (Figure 23 *e, g, h*). We have not yet determined if these profiles represent dendritic cells, macrophages or both. Some strongly stained cells within the airways, presumably alveolar macrophages, were evident (*, Figure 23 *h*). DEC-205 was present in the epithelium of all the small airways (*arrows*, Figure 23 *e, h*). In contrast, anti-MHC class II did not stain the airway epithelium, but did stain cells surrounding the airways (Figure 23 *f, arrowheads*).

Bone marrow. When bone marrow was extruded from the femur as an intact plug and sectioned, a lacy pattern of DEC-205 stain was evident throughout the plug, presumably on marrow stromal cells (*arrows*, Figure 23 *i*). Most of the dark staining of round cells represented background staining of eosinophils, which express endogenous peroxidases. It was evident in the absence of any antibody (not shown).

Upper gastrointestinal tract. The oral epithelium of the tongue served as an example of a stratified squamous epithelium. Some DEC-205 positive profiles, presumably Langerhans cells, were found suprabasally (*arrows*, Figure 23 *j*). Anti-MHC class II antibodies stained these intraepithelial dendritic cells more frequently
5 and/or more intensely, and in addition stained many subepithelial profiles in the upper dermis (not shown).

Lower gastrointestinal tract. Strong staining was observed on the columnar epithelia of the small and large bowel. The staining was much greater on apical
10 villi than on crypt epithelium (Figure 23, *k-l*). In the best sections, staining was also stronger along the basal surfaces than the apices of individual epithelial cells. Many cells within the lamina propria of the villi stained darkly, but this staining was again due to endogenous peroxidase within eosinophils.

15 *Liver.* No staining for DEC-205 was apparent, except for rare profiles in the portal triads (not shown).

Heart. No staining for DEC-205 was apparent (not shown).

20 *Kidney.* No strong expression of DEC-205 was noted, although some very weak DEC-205 staining was observed on scattered cortical tubules (not shown).

Distribution of DEC-205 by multiple-organ immunoblotting. To examine the tissue distribution of the DEC-205 protein itself, TRIzol protein extracts of several
25 different organs (Methods) were immunoblotted with both monoclonal NLDC-145 IgG (Figure 24A) and polyclonal anti-DEC-205 IgG (not shown). The quantities of organ lysate protein applied to each lane were normalized to one another in terms of protein load (50 μ g per lane). Staining for the lysosomal membrane marker LAMP-1 (Chen et al., 1985, Arch. Biochem. Biophys. 239:574) revealed that
30 comparable numbers of lysosomes were represented in each lane (Figure 24B). Among lymphoid tissues, the signal for DEC-205 was greatest in the thymus, and

was stronger in bone marrow and lymph nodes than in the spleen. These findings corresponded closely to the histologic staining levels described above. In nonlymphoid tissues, strong signals comparable to thymus were evident in lung and intestine (Figure 24A). Liver, kidney and brain extracts contained only trace levels
5 of DEC-205.

Discussion

Using an improved donkey F(ab')₂ secondary anti-rat Ig reagent to increase the sensitivity of antigen detection, we have shown that the NLDC-145 monoclonal
10 antibody reacts with many more tissues than previously apparent. In particular, clear staining was observed on brain capillaries, bone marrow stroma, the epithelia of intestinal villi and pulmonary airways, and B cells in the follicles of all peripheral lymphoid tissues. These newly-recognized depots of DEC-205 antigen did not stain as strongly as tissues that were originally noted to express the DEC-
15 205 protein, *i.e.*, the cortical epithelium of the thymus, and the dendritic cells in the T cell areas of peripheral lymphoid organs.

The tissue distribution of the DEC-205 protein was confirmed with a polyclonal antibody raised to purified DEC-205. Both F(ab')₂ and intact IgG forms of the
20 rabbit antibody gave patterns of staining that were similar to those obtained with monoclonal NLDC-145. The tissue distribution was also monitored by immunoblotting with both monoclonal and polyclonal reagents, and the relative levels of expression in multiple organs corresponded to the relative intensities of immunohistochemical staining seen on tissue sections. An exception is that trace
25 amounts of DEC-205 protein were evident in extracts from organs like liver, heart, and kidney where it was difficult to appreciate discrete DEC-205 positive cells. This suggests that very small amounts of the protein may be present in many cell types, but at levels too low to permit histologic visualization.

EXAMPLE: SEQUENCE OF HUMAN DEC-205

Human *dec* cDNA was cloned using a 300 base-pair probe derived from the 3' coding sequence of murine *dec* cDNA. The human cDNA was obtained from a B lymphoma library, using high stringency hybridization conditions (0.1 SSC, 65°C). The sequence of the human DEC-205 gene was determined, and is shown in SEQ ID NO:7. The deduced amino acid sequence is shown in SEQ ID NO:8. The deduced sequence in SEQ ID NO:8 includes putative segments after the stop site at after amino acid number 1743; these are irrelevant and may be ignored.

10

The human deduced amino acid sequence and nucleotide sequence were compared with their murine homologs, as shown in Figure 25A and 25B, respectively. This comparison shows a high degree of sequence similarity or identity for the entire length of both proteins and protein coding regions of the genes (cDNAs).

15

The present invention is not to be limited in scope by the specific embodiments described herein since such embodiments are intended as but single illustrations of one aspect of the invention and any embodiments which are functionally equivalent are within the scope of this invention. It should be further understood that all molecular weight and nucleotide base pair sizes given for nucleotides are approximate and are used for the purpose of description. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference herein in their entireties.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: STEINMAN, RALPH A
NUSSENZWEIG, MICHEL C
SWIGGARD, WILLIAM J
JIANG, WANPING
- (ii) TITLE OF INVENTION: IDENTIFICATION OF DEC, A RECEPTOR WITH
C-TYPE LECTIN DOMAINS, NUCLEIC ACIDS ENCODING DEC, AND
USES THEREOF
- (iii) NUMBER OF SEQUENCES: 8
- (iv) CORRESPONDENCE ADDRESS:
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- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) PRIOR APPLICATION DATA:
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 - (B) FILING DATE: 31-JAN-1996
 - (C) CLASSIFICATION:
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- (x) Note: In all the amino acid sequences below, "Xaa" stands for one
of the three stop codons.

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
 - (A) DESCRIPTION: cytoplasmic domain of human DEC-205

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg His Arg Leu His Leu Ala Gly Phe Ser Ser Val Arg Tyr Ala Gln
1 5 10 15

Gly Val Asn Glu Asp Glu Ile Met Leu Pro Ser Phe His Asp.
20 25 30

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(A) DESCRIPTION: human DEC-205, peptide N1

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ser Glu Ser Ser Gly Asn Asp Pro Phe Thr Ile Val His Glu Asn Thr
1 5 10 15

Gly Lys Cys Ile Gln Pro Leu Phe Asp
20 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1723 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(A) DESCRIPTION: predicted amino acid sequence of DEC-205

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Arg Thr Gly Arg Val Thr Pro Gly Leu Ala Ala Gly Leu Leu Leu
1 5 10 15

Leu Leu Leu Arg Ser Phe Gly Leu Val Glu Pro Ser Glu Ser Ser Gly
20 25 30

Asn Asp Pro Phe Thr Ile Val His Glu Asn Thr Gly Lys Cys Ile Gln
35 40 45

Pro Leu Ser Asp Trp Val Val Ala Gln Asp Cys Ser Gly Thr Asn Asn
50 55 60

94

Met	Leu	Trp	Lys	Trp	Val	Ser	Gln	His	Arg	Leu	Phe	His	Leu	Glu	Ser	65	70	75	80
Gln	Lys	Cys	Leu	Gly	Leu	Asp	Ile	Thr	Lys	Ala	Thr	Asp	Asn	Leu	Arg	85	90	95	
Met	Phe	Ser	Cys	Asp	Ser	Thr	Val	Met	Leu	Trp	Trp	Lys	Cys	Glu	His	100	105	110	
His	Ser	Leu	Tyr	Thr	Ala	Ala	Gln	Tyr	Arg	Leu	Ala	Leu	Lys	Asp	Gly	115	120	125	
Tyr	Ala	Val	Ala	Asn	Thr	Asn	Thr	Ser	Asp	Val	Trp	Lys	Lys	Gly	Gly	130	135	140	
Ser	Glu	Glu	Asn	Leu	Cys	Ala	Gln	Pro	Tyr	His	Glu	Ile	Tyr	Thr	Arg	145	150	155	160
Asp	Gly	Asn	Ser	Tyr	Gly	Arg	Pro	Cys	Glu	Phe	Pro	Phe	Leu	Ile	Gly	165	170	175	
Glu	Thr	Trp	Tyr	His	Asp	Cys	Ile	His	Asp	Glu	Asp	His	Ser	Gly	Pro	180	185	190	
Trp	Cys	Ala	Thr	Thr	Leu	Ser	Tyr	Glu	Tyr	Asp	Gln	Lys	Trp	Gly	Ile	195	200	205	
Cys	Leu	Leu	Pro	Glu	Ser	Gly	Cys	Glu	Gly	Asn	Trp	Glu	Lys	Asn	Glu	210	215	220	
Gln	Ile	Gly	Ser	Cys	Tyr	Gln	Phe	Asn	Asn	Gln	Glu	Ile	Leu	Ser	Trp	225	230	235	240
Lys	Glu	Ala	Tyr	Val	Ser	Cys	Gln	Asn	Gln	Gly	Ala	Asp	Leu	Leu	Ser	245	250	255	
Ile	His	Ser	Ala	Ala	Glu	Leu	Ala	Tyr	Ile	Thr	Gly	Lys	Glu	Asp	Ile	260	265	270	
Ala	Arg	Leu	Val	Trp	Leu	Gly	Leu	Asn	Gln	Leu	Tyr	Ser	Ala	Arg	Gly	275	280	285	
Trp	Glu	Trp	Ser	Asp	Phe	Arg	Pro	Leu	Lys	Phe	Leu	Asn	Trp	Asp	Pro	290	295	300	
Gly	Thr	Pro	Val	Ala	Pro	Val	Ile	Gly	Gly	Ser	Ser	Cys	Ala	Arg	Met	305	310	315	320
Asp	Thr	Glu	Ser	Gly	Leu	Trp	Gln	Ser	Val	Ser	Cys	Glu	Ser	Gln	Gln	325	330	335	
Pro	Tyr	Val	Cys	Lys	Lys	Pro	Leu	Asn	Asn	Thr	Leu	Glu	Leu	Pro	Asp	340	345	350	
Val	Trp	Thr	Tyr	Thr	Asp	Thr	His	Cys	His	Val	Gly	Trp	Leu	Pro	Asn	355	360	365	
Asn	Gly	Phe	Cys	Tyr	Leu	Leu	Ala	Asn	Glu	Ser	Ser	Ser	Trp	Asp	Ala	370	375	380	
Ala	His	Leu	Lys	Cys	Lys	Ala	Phe	Gly	Ala	Asp	Leu	Ile	Ser	Met	His	385	390	395	400
Ser	Leu	Ala	Asp	Val	Glu	Val	Val	Val	Thr	Lys	Leu	His	Asn	Gly	Asp	405	410	415	

Val Lys Lys Glu Ile Trp Thr Gly Leu Lys Asn Thr Asn Ser Pro Ala
 420 425 430
 Leu Phe Gln Trp Ser Asp Gly Thr Glu Val Thr Leu Thr Tyr Trp Asn
 435 440 445
 Glu Asn Glu Pro Ser Val Pro Phe Asn Lys Thr Pro Asn Cys Val Ser
 450 455 460
 Tyr Leu Gly Lys Leu Gly Gln Trp Lys Val Gln Ser Cys Glu Lys Lys
 465 470 475 480
 Leu Arg Tyr Val Cys Lys Lys Lys Gly Glu Ile Thr Lys Asp Ala Glu
 485 490 495
 Ser Asp Lys Leu Cys Pro Pro Asp Glu Gly Trp Lys Arg His Gly Glu
 500 505 510
 Thr Cys Tyr Lys Ile Tyr Glu Lys Glu Ala Pro Phe Gly Thr Asn Cys
 515 520 525
 Asn Leu Thr Ile Thr Ser Arg Phe Glu Gln Glu Phe Leu Asn Tyr Met
 530 535 540
 Met Lys Asn Tyr Asp Lys Ser Leu Arg Lys Tyr Phe Trp Thr Gly Leu
 545 550 555 560
 Arg Asp Pro Asp Ser Arg Gly Glu Tyr Ser Trp Ala Val Ala Gln Gly
 565 570 575
 Val Lys Gln Ala Val Thr Phe Ser Asn Trp Asn Phe Leu Glu Pro Ala
 580 585 590
 Ser Pro Gly Gly Cys Val Ala Met Ser Thr Gly Lys Thr Leu Gly Lys
 595 600 605
 Trp Glu Val Lys Asn Cys Arg Ser Phe Arg Ala Leu Ser Ile Cys Lys
 610 615 620
 Lys Val Ser Glu Pro Gln Glu Pro Glu Glu Ala Ala Pro Lys Pro Asp
 625 630 635 640
 Asp Pro Cys Pro Glu Gly Trp His Thr Phe Pro Ser Ser Leu Ser Cys
 645 650 655
 Tyr Lys Val Phe His Ile Glu Arg Ile Val Arg Lys Arg Asn Trp Glu
 660 665 670
 Glu Ala Glu Arg Phe Cys Gln Ala Leu Gly Ala His Leu Pro Ser Phe
 675 680 685
 Ser Arg Arg Glu Glu Ile Lys Asp Phe Val His Leu Leu Lys Asp Gln
 690 695 700
 Phe Ser Gly Gln Arg Trp Leu Trp Ile Gly Leu Asn Lys Arg Ser Pro
 705 710 715 720
 Asp Leu Gln Gly Ser Trp Gln Trp Ser Asp Arg Thr Pro Val Ser Ala
 725 730 735
 Val Met Met Glu Pro Glu Phe Gln Gln Asp Phe Asp Ile Arg Asp Cys
 740 745 750
 Ala Ala Ile Lys Val Leu Asp Val Pro Trp Arg Arg Val Trp His Leu
 755 760 765

Tyr Glu Asp Lys Asp Tyr Ala Tyr Trp Lys Pro Phe Ala Cys Asp Ala
 770 775 780
 Lys Leu Glu Trp Val Cys Gln Ile Pro Lys Gly Ser Thr Pro Gln Met
 785 790 795 800
 Pro Asp Trp Tyr Asn Pro Glu Arg Thr Gly Ile His Gly Pro Pro Val
 805 810 815
 Ile Ile Glu Gly Ser Glu Tyr Trp Phe Val Ala Asp Pro His Leu Asn
 820 825 830
 Tyr Glu Glu Ala Val Leu Tyr Cys Ala Ser Asn His Ser Phe Leu Ala
 835 840 845
 Thr Ile Thr Ser Phe Thr Gly Leu Lys Ala Ile Lys Asn Lys Leu Ala
 850 855 860
 Asn Ile Ser Gly Glu Glu Gln Lys Trp Trp Val Lys Thr Ser Glu Asn
 865 870 875 880
 Pro Ile Asp Arg Tyr Phe Leu Gly Ser Arg Arg Arg Leu Trp His His
 885 890 895
 Phe Pro Met Thr Phe Gly Asp Glu Cys Leu His Met Ser Ala Lys Thr
 900 905 910
 Trp Leu Val Asp Leu Ser Lys Arg Ala Asp Cys Asn Ala Lys Leu Pro
 915 920 925
 Phe Ile Cys Glu Arg Tyr Asn Val Ser Ser Leu Glu Lys Tyr Ser Pro
 930 935 940
 Asp Pro Ala Ala Lys Val Gln Cys Thr Glu Lys Trp Ile Pro Phe Gln
 945 950 955 960
 Asn Lys Cys Phe Leu Lys Val Asn Ser Gly Pro Val Thr Phe Ser Gln
 965 970 975
 Ala Ser Gly Ile Cys His Ser Tyr Gly Gly Thr Leu Pro Ser Val Leu
 980 985 990
 Ser Arg Gly Glu Gln Asp Phe Ile Ile Ser Leu Leu Pro Glu Met Glu
 995 1000 1005
 Ala Ser Leu Trp Ile Gly Leu Arg Trp Thr Ala Tyr Glu Arg Ile Asn
 1010 1015 1020
 Arg Trp Thr Asp Asn Arg Glu Leu Thr Tyr Ser Asn Phe His Pro Leu
 1025 1030 1035 1040
 Leu Val Gly Arg Arg Leu Ser Ile Pro Thr Asn Phe Phe Asp Asp Glu
 1045 1050 1055
 Ser His Phe His Cys Ala Leu Ile Leu Asn Leu Lys Lys Ser Pro Leu
 1060 1065 1070
 Thr Gly Thr Trp Asn Phe Thr Ser Cys Ser Glu Arg His Ser Leu Ser
 1075 1080 1085
 Leu Cys Gln Lys Tyr Ser Glu Thr Glu Asp Gly Gln Pro Trp Glu Asn
 1090 1095 1100
 Thr Ser Lys Thr Val Lys Tyr Leu Asn Asn Leu Tyr Lys Ile Ile Ser
 1105 1110 1115 1120

Lys Pro Leu Thr Trp His Gly Ala Leu Lys Glu Cys Met Lys Glu Lys
 1125 1130 1135
 Met Arg Leu Val Ser Ile Thr Asp Pro Tyr Gln Gln Ala Phe Leu Ala
 1140 1145 1150
 Val Gln Ala Thr Leu Arg Asn Ser Ser Phe Trp Ile Gly Leu Ser Ser
 1155 1160 1165
 Gln Asp Asp Glu Leu Asn Phe Gly Trp Ser Asp Gly Lys Arg Leu Gln
 1170 1175 1180
 Phe Ser Asn Trp Ala Gly Ser Asn Glu Gln Leu Asp Asp Cys Val Ile
 1185 1190 1195 1200
 Leu Asp Thr Asp Gly Phe Trp Lys Thr Ala Asp Cys Asp Asp Asn Gln
 1205 1210 1215
 Pro Gly Ala Ile Cys Tyr Tyr Pro Gly Asn Glu Thr Glu Glu Glu Val
 1220 1225 1230
 Arg Ala Leu Asp Thr Ala Lys Cys Pro Ser Pro Val Gln Ser Thr Pro
 1235 1240 1245
 Trp Ile Pro Phe Gln Asn Ser Cys Tyr Asn Phe Met Ile Thr Asn Asn
 1250 1255 1260
 Arg His Lys Thr Val Thr Pro Glu Glu Val Gln Ser Thr Cys Glu Lys
 1265 1270 1275 1280
 Leu His Pro Lys Ala His Ser Leu Ser Ile Arg Asn Glu Glu Glu Asn
 1285 1290 1295
 Thr Phe Val Val Glu Gln Leu Leu Tyr Phe Asn Tyr Ile Ala Ser Trp
 1300 1305 1310
 Val Met Leu Gly Ile Thr Tyr Glu Asn Asn Ser Leu Met Trp Phe Asp
 1315 1320 1325
 Lys Thr Ala Leu Ser Tyr Thr His Trp Arg Thr Gly Arg Pro Thr Val
 1330 1335 1340
 Lys Asn Gly Lys Phe Leu Ala Gly Leu Ser Thr Asp Gly Phe Trp Asp
 1345 1350 1355 1360
 Ile Gln Ser Phe Asn Val Ile Glu Glu Thr Leu His Phe Tyr Gln His
 1365 1370 1375
 Ser Ile Ser Ala Cys Lys Ile Glu Met Val Asp Tyr Glu Asp Lys His
 1380 1385 1390
 Asn Gly Thr Leu Pro Gln Phe Ile Pro Tyr Lys Asp Gly Val Tyr Ser
 1395 1400 1405
 Val Ile Gln Lys Lys Val Thr Trp Tyr Glu Ala Leu Asn Ala Cys Ser
 1410 1415 1420
 Gln Ser Gly Gly Glu Leu Ala Ser Val His Asn Pro Asn Gly Lys Leu
 1425 1430 1435 1440
 Phe Leu Glu Asp Ile Val Asn Arg Asp Gly Phe Pro Leu Trp Val Gly
 1445 1450 1455
 Leu Ser Ser His Asp Gly Ser Glu Ser Ser Phe Glu Trp Ser Asp Gly
 1460 1465 1470

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Arg Ala Phe Asp Tyr Val Pro Trp Gln Ser Leu Gln Ser Pro Gly Asp
 1475 1480 1485
 Cys Val Val Leu Tyr Pro Lys Gly Ile Trp Arg Arg Glu Lys Cys Leu
 1490 1495 1500
 Ser Val Lys Asp Gly Ala Ile Cys Tyr Lys Pro Thr Lys Asp Lys Lys
 1505 1510 1515 1520
 Leu Ile Phe His Val Lys Ser Ser Lys Cys Pro Val Ala Lys Arg Asp
 1525 1530 1535
 Gly Pro Gln Trp Val Gln Tyr Gly Gly His Cys Tyr Ala Ser Asp Gln
 1540 1545 1550
 Val Leu His Ser Phe Ser Glu Ala Lys Gln Val Cys Gln Glu Leu Asp
 1555 1560 1565
 His Ser Ala Thr Val Val Thr Ile Ala Asp Glu Asn Glu Asn Lys Phe
 1570 1575 1580
 Val Ser Arg Leu Met Arg Glu Asn Tyr Asn Ile Thr Met Arg Val Trp
 1585 1590 1595 1600
 Leu Gly Leu Ser Gln His Ser Leu Asp Gln Ser Trp Ser Trp Leu Asp
 1605 1610 1615
 Gly Leu Asp Val Thr Phe Val Lys Trp Glu Asn Lys Thr Lys Asp Gly
 1620 1625 1630
 Asp Gly Lys Cys Ser Ile Leu Ile Ala Ser Asn Glu Thr Trp Arg Lys
 1635 1640 1645
 Val His Cys Ser Arg Gly Tyr Ala Arg Ala Val Cys Lys Ile Pro Leu
 1650 1655 1660
 Ser Pro Asp Tyr Thr Gly Ile Ala Ile Leu Phe Ala Val Leu Cys Leu
 1665 1670 1675 1680
 Leu Gly Leu Ile Ser Leu Ala Ile Trp Phe Leu Leu Gln Arg Ser His
 1685 1690 1695
 Ile Arg Trp Thr Gly Phe Ser Ser Val Arg Tyr Glu His Gly Thr Asn
 1700 1705 1710
 Glu Asp Glu Val Met Leu Pro Ser Phe His Asp
 1715 1720

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1462 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
 - (A) DESCRIPTION: bovine PLA2 receptor
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: bovine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Pro	Leu	Leu	Ser	Leu	Ser	Leu	Leu	Leu	Leu	Leu	Gln	Val	Pro	1	5	10	15
Ala	Gly	Ser	Ala	Glu	Thr	Ala	Ala	Trp	Ala	Val	Thr	Pro	Glu	Arg	Leu	20	25	30
Arg	Glu	Trp	Gln	Asp	Lys	Gly	Ile	Phe	Ile	Ile	Gln	Ser	Glu	Asn	Leu	35	40	45
Glu	Lys	Cys	Ile	Gln	Ala	Ser	Lys	Ser	Thr	Leu	Thr	Leu	Glu	Asn	Cys	50	55	60
Lys	Pro	Pro	Asn	Lys	Tyr	Met	Leu	Trp	Lys	Trp	Val	Ser	Asn	His	Arg	65	70	75
Leu	Phe	Asn	Ile	Gly	Gly	Ser	Gly	Cys	Leu	Gly	Leu	Asn	Val	Ser	Ser	85	90	95
Pro	Glu	Gln	Pro	Leu	Ser	Ile	Tyr	Glu	Cys	Asp	Ser	Thr	His	Val	Ser	100	105	110
Leu	Lys	Trp	His	Cys	Asn	Lys	Lys	Thr	Ile	Thr	Gly	Pro	Leu	Gln	Tyr	115	120	125
Leu	Val	Gln	Val	Lys	Gln	Asp	Asn	Thr	Leu	Val	Ala	Ser	Arg	Lys	Tyr	130	135	140
Leu	His	Lys	Trp	Val	Ser	Tyr	Met	Ser	Gly	Gly	Gly	Gly	Ile	Cys	Asp	145	150	155
Tyr	Leu	His	Lys	Asp	Leu	Tyr	Thr	Ile	Lys	Gly	Asn	Ala	His	Gly	Thr	165	170	175
Pro	Cys	Met	Phe	Pro	Phe	Gln	Tyr	Asn	Gln	Gln	Trp	His	His	Glu	Cys	180	185	190
Thr	Arg	Glu	Gly	Arg	Glu	Asp	Asn	Leu	Leu	Trp	Cys	Ala	Thr	Thr	Ser	195	200	205
Arg	Tyr	Glu	Arg	Asp	Glu	Lys	Trp	Gly	Phe	Cys	Pro	Asp	Pro	Thr	Ser	210	215	220
Thr	Glu	Val	Gly	Cys	Asp	Ala	Val	Trp	Glu	Lys	Asp	Leu	His	Ser	Arg	225	230	235
Ile	Cys	Tyr	Gln	Phe	Asn	Leu	Leu	Ser	Ser	Leu	Ser	Trp	Ser	Glu	Ala	245	250	255
His	Ser	Ser	Cys	Gln	Met	Gln	Gly	Ala	Ala	Leu	Leu	Ser	Ile	Ala	Asp	260	265	270
Glu	Thr	Glu	Glu	Asn	Phe	Val	Arg	Lys	His	Leu	Gly	Ser	Glu	Ala	Val	275	280	285
Glu	Val	Trp	Met	Gly	Leu	Asn	Gln	Leu	Asp	Glu	Asp	Ala	Gly	Trp	Gln	290	295	300
Trp	Ser	Asp	Arg	Thr	Pro	Leu	Asn	Tyr	Leu	Asn	Trp	Lys	Pro	Glu	Ile	305	310	315
Asn	Phe	Glu	Pro	Phe	Val	Glu	Tyr	His	Cys	Gly	Thr	Phe	Asn	Ala	Phe	325	330	335

100

Met	Pro	Lys	Ala	Trp	Lys	Ser	Arg	Asp	Cys	Glu	Ser	Thr	Leu	Pro	Tyr		
			340					345					350				
Val	Cys	Lys	Lys	Tyr	Leu	Asn	Pro	Thr	Asp	His	Gly	Val	Val	Glu	Lys		
		355					360					365					
Asp	Ala	Trp	Lys	Tyr	Tyr	Ala	Thr	His	Cys	Glu	Pro	Gly	Trp	Asn	Pro		
	370					375					380						
His	Asn	Arg	Asn	Cys	Tyr	Lys	Leu	Gln	Lys	Glu	Lys	Lys	Thr	Trp	Asn		
385					390					395					400		
Glu	Ala	Leu	Gln	Ser	Cys	Gln	Ser	Asn	Asn	Ser	Val	Leu	Thr	Asp	Ile		
				405					410					415			
Thr	Ser	Leu	Ala	Glu	Val	Glu	Phe	Leu	Val	Thr	Leu	Leu	Gly	Asp	Glu		
			420					425					430				
Asn	Ala	Ser	Glu	Thr	Trp	Ile	Gly	Leu	Ser	Ser	His	Lys	Ile	Pro	Val		
		435					440					445					
Ser	Phe	Glu	Trp	Ser	Asn	Gly	Ser	Ser	Val	Thr	Phe	Thr	Asn	Trp	His		
	450					455					460						
Thr	Leu	Glu	Pro	His	Ile	Phe	Pro	Asn	Arg	Ser	Gln	Leu	Cys	Val	Ser		
465					470					475					480		
Ala	Glu	Gln	Ser	Glu	Gly	His	Trp	Lys	Val	Lys	Asn	Cys	Glu	Glu	Thr		
				485					490					495			
Leu	Phe	Tyr	Leu	Cys	Lys	Lys	Thr	His	Leu	Val	Leu	Ser	Asp	Thr	Glu		
			500					505					510				
Ser	Gly	Cys	Gln	Lys	Gly	Trp	Glu	Arg	His	Gly	Lys	Phe	Cys	Tyr	Lys		
		515					520					525					
Ile	Asp	Thr	Val	Leu	Arg	Ser	Phe	Asp	His	Ala	Ser	Ser	Gly	Tyr	Tyr		
	530					535					540						
Cys	Pro	Pro	Ala	Leu	Ile	Thr	Ile	Thr	Ser	Arg	Phe	Glu	Gln	Ala	Phe		
545					550					555					560		
Ile	Thr	Ser	Leu	Ile	Ser	Ser	Val	Val	Lys	Thr	Lys	Asp	Thr	Tyr	Phe		
				565					570					575			
Trp	Ile	Ala	Leu	Gln	Asp	Gln	Asn	Asn	Thr	Gly	Glu	Tyr	Thr	Trp	Lys		
			580					585						590			
Thr	Ala	Gly	Gln	Gln	Leu	Glu	Pro	Val	Lys	Tyr	Thr	His	Trp	Asn	Thr		
		595					600					605					
Arg	Gln	Pro	Arg	Tyr	Ser	Gly	Gly	Cys	Val	Val	Met	Arg	Gly	Arg	Ser		
		610				615					620						
His	Pro	Gly	Arg	Trp	Glu	Val	Arg	Asp	Cys	Arg	His	Phe	Lys	Ala	Met		
625					630					635					640		
Ser	Leu	Cys	Lys	Gln	Pro	Val	Glu	Asn	Arg	Glu	Lys	Thr	Lys	Gln	Glu		
				645					650					655			
Glu	Gly	Trp	Pro	Phe	His	Pro	Cys	Tyr	Leu	Asp	Trp	Glu	Ser	Glu	Pro		
			660					665					670				
Gly	Leu	Ala	Ser	Cys	Phe	Lys	Val	Phe	His	Ser	Glu	Lys	Val	Leu	Met		
		675					680					685					

101

Lys Arg Thr Trp Arg Gln Ala Glu Glu Phe Cys Glu Glu Phe Gly Ala
 690 695 700
 His Leu Ala Ser Phe Ala His Ile Glu Glu Glu Asn Phe Val Asn Glu
 705 710 715 720
 Leu Leu His Ser Lys Phe Asn Arg Thr Glu Glu Arg Gln Phe Trp Ile
 725 730 735
 Gly Phe Asn Lys Arg Asn Pro Leu Asn Ala Gly Ser Trp Glu Trp Ser
 740 745 750
 Asp Gly Thr Pro Val Val Ser Ser Phe Leu Asp Asn Ser Tyr Phe Gly
 755 760 765
 Glu Asp Ala Arg Asn Cys Ala Val Tyr Lys Ala Asn Lys Thr Leu Leu
 770 775 780
 Pro Ser Tyr Cys Gly Ser Lys Arg Glu Trp Ile Cys Lys Ile Pro Arg
 785 790 795 800
 Asp Val Arg Pro Lys Val Pro Pro Trp Tyr Gln Tyr Asp Ala Pro Trp
 805 810 815
 Leu Phe Tyr Gln Asp Ala Glu Tyr Leu Phe His Ile Ser Ala Ser Glu
 820 825 830
 Trp Ser Ser Phe Glu Phe Val Cys Gly Trp Leu Arg Ser Asp Ile Leu
 835 840 845
 Thr Ile His Ser Ala His Glu Gln Glu Phe Ile His Ser Lys Ile Arg
 850 855 860
 Ala Leu Ser Lys Tyr Gly Val Asn Trp Trp Ile Gly Leu Arg Glu Glu
 865 870 875 880
 Arg Ala Ser Asp Glu Phe Arg Trp Arg Asp Gly Ser Pro Val Ile Tyr
 885 890 895
 Gln Asn Trp Asp Lys Gly Lys Glu Arg Ser Met Gly Leu Asn Glu Ser
 900 905 910
 Gln Arg Cys Gly Phe Ile Ser Ser Ile Thr Gly Leu Trp Ala Ser Glu
 915 920 925
 Glu Cys Ser Ile Ser Met Pro Ser Ile Cys Lys Arg Lys Lys Val Trp
 930 935 940
 Val Ile Glu Lys Lys Lys Asp Ile Pro Lys Gln His Gly Thr Cys Pro
 945 950 955 960
 Lys Gly Trp Leu Tyr Phe Asp Tyr Lys Cys Leu Leu Leu Lys Ile Pro
 965 970 975
 Glu Gly Pro Ser Asp Trp Lys Asn Trp Thr Ser Ala Gln Asp Phe Cys
 980 985 990
 Val Glu Glu Gly Gly Thr Leu Val Ala Ile Glu Asn Glu Val Glu Gln
 995 1000 1005
 Ala Phe Ile Thr Met Asn Leu Phe Gly His Thr Thr Asn Val Trp Ile
 1010 1015 1020
 Gly Leu Gln Asp Asp Asp Tyr Glu Lys Trp Leu Asn Gly Arg Pro Val
 1025 1030 1035 1040

102

Ser Tyr Ser Asn Trp Ser Pro Phe Asp Thr Lys Asn Ile Pro Asn His
 1045 1050 1055
 Asn Thr Thr Glu Val Gln Lys Arg Ile Pro Leu Cys Gly Leu Leu Ser
 1060 1065 1070
 Asn Asn Pro Asn Phe His Phe Thr Gly Lys Trp Tyr Phe Asp Cys Arg
 1075 1080 1085
 Glu Gly Tyr Gly Phe Val Cys Glu Lys Met Gln Asp Ala Ser Gly His
 1090 1095 1100
 Ser Ile Asn Thr Ser Asp Met Tyr Pro Ile Pro Asn Thr Leu Glu Tyr
 1105 1110 1115 1120
 Gly Asn Arg Thr Tyr Lys Ile Ile Asn Ala Asn Met Thr Trp Tyr Thr
 1125 1130 1135
 Ala Leu Lys Thr Cys Leu Met His Gly Ala Glu Leu Ala Ser Ile Thr
 1140 1145 1150
 Asp Gln Tyr His Gln Ser Phe Leu Thr Val Ile Leu Asn Arg Val Gly
 1155 1160 1165
 Tyr Ala His Trp Ile Gly Leu Phe Thr Glu Asp Asn Gly Leu Ser Phe
 1170 1175 1180
 Asp Trp Ser Asp Gly Thr Lys Ser Ser Phe Thr Phe Trp Lys Asp Asp
 1185 1190 1195 1200
 Glu Ser Ser Phe Leu Gly Asp Cys Val Phe Ala Asp Thr Ser Gly Arg
 1205 1210 1215
 Trp Ser Ser Thr Ala Cys Glu Ser Tyr Leu Gln Gly Ala Ile Cys Gln
 1220 1225 1230
 Val Pro Thr Glu Thr Arg Leu Ser Gly Arg Leu Glu Leu Cys Ser Glu
 1235 1240 1245
 Thr Ser Ile Pro Trp Ile Lys Phe Lys Ser Asn Cys Tyr Ser Phe Ser
 1250 1255 1260
 Thr Val Leu Glu Ser Thr Ser Phe Glu Ala Ala His Glu Phe Cys Lys
 1265 1270 1275 1280
 Lys Lys Gly Ser Asn Leu Leu Thr Ile Lys Asp Glu Ala Glu Asn Ser
 1285 1290 1295
 Phe Leu Leu Glu Glu Leu Leu Ala Phe Arg Ser Ser Val Gln Met Ile
 1300 1305 1310
 Trp Leu Asn Ala Gln Phe Asp Gly Asp Asn Glu Thr Ile Lys Trp Phe
 1315 1320 1325
 Asp Gly Thr Pro Thr Asp Gln Ser Asn Trp Gly Ile Arg Lys Pro Glu
 1330 1335 1340
 Val Tyr His Phe Lys Pro His Leu Cys Val Ala Leu Arg Ile Pro Glu
 1345 1350 1355 1360
 Gly Val Trp Gln Leu Ser Ser Cys Gln Asp Lys Lys Gly Phe Ile Cys
 1365 1370 1375
 Lys Met Glu Ala Asp Ile His Thr Val Lys Lys His Pro Gly Lys Gly
 1380 1385 1390

103

Pro Ser His Ser Val Ile Pro Leu Thr Val Ala Leu Thr Leu Leu Val
 1395 1400 1405

Ile Leu Ala Ile Ser Thr Leu Ser Phe Cys Met Tyr Lys His Ser His
 1410 1415 1420

Ile Ile Phe Gly Arg Leu Ala Gln Phe Arg Asn Pro Tyr Tyr Pro Ser
 1425 1430 1435 1440

Ala Asn Phe Ser Thr Val His Leu Glu Glu Asn Ile Leu Ile Ser Asp
 1445 1450 1455

Leu Glu Lys Asn Asp Gln
 1460

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1457 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- (A) DESCRIPTION: human macrophage mannose receptor

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: human

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Arg Leu Pro Leu Leu Leu Val Phe Ala Ser Val Ile Pro Gly Ala
 1 5 10 15

Val Leu Leu Leu Asp Thr Arg Gln Phe Leu Ile Tyr Asn Glu Asp His
 20 25 30

Lys Arg Cys Val Asp Ala Val Ser Pro Ser Ala Val Gln Thr Ala Ala
 35 40 45

Cys Asn Gln Asp Ala Glu Ser Gln Lys Phe Arg Trp Val Ser Glu Ser
 50 55 60

Gln Ile Met Ser Val Ala Phe Lys Leu Cys Leu Gly Val Pro Ser Lys
 65 70 75 80

Thr Asp Trp Val Ala Ile Thr Leu Tyr Ala Cys Asp Ser Lys Ser Glu
 85 90 95

Phe Gln Lys Trp Glu Cys Lys Asn Asp Thr Leu Leu Gly Ile Lys Gly
 100 105 110

Glu Asp Leu Phe Phe Asn Tyr Gly Asn Arg Gln Glu Lys Asn Ile Met
 115 120 125

Leu Tyr Lys Gly Ser Gly Leu Trp Ser Arg Trp Lys Ile Tyr Gly Thr
 130 135 140

Thr Asp Asn Leu Cys Ser Arg Gly Tyr Glu Ala Met Tyr Thr Leu Leu
 145 150 155 160

104

Gly	Asn	Ala	Asn	Gly	Ala	Thr	Cys	Ala	Phe	Pro	Phe	Lys	Phe	Glu	Asn
				165					170					175	
Lys	Trp	Tyr	Ala	Asp	Cys	Thr	Ser	Ala	Gly	Arg	Ser	Asp	Gly	Trp	Leu
			180					185					190		
Trp	Cys	Gly	Thr	Thr	Thr	Asp	Tyr	Asp	Thr	Asp	Lys	Leu	Phe	Gly	Tyr
		195					200					205			
Cys	Pro	Leu	Lys	Phe	Glu	Gly	Ser	Glu	Ser	Leu	Trp	Asn	Lys	Asp	Pro
	210					215					220				
Leu	Thr	Ser	Val	Ser	Tyr	Gln	Ile	Asn	Ser	Lys	Ser	Ala	Leu	Thr	Trp
225					230					235					240
His	Gln	Ala	Arg	Lys	Ser	Cys	Gln	Gln	Gln	Asn	Ala	Glu	Leu	Leu	Ser
				245					250					255	
Ile	Thr	Glu	Ile	His	Glu	Gln	Thr	Tyr	Leu	Thr	Gly	Leu	Thr	Ser	Ser
			260					265					270		
Leu	Thr	Ser	Gly	Leu	Trp	Ile	Gly	Leu	Asn	Ser	Leu	Ser	Phe	Asn	Ser
		275					280					285			
Gly	Trp	Gln	Trp	Ser	Asp	Arg	Ser	Pro	Phe	Arg	Tyr	Leu	Asn	Trp	Leu
	290					295					300				
Pro	Gly	Ser	Pro	Ser	Ala	Glu	Pro	Gly	Lys	Ser	Cys	Val	Ser	Leu	Asn
305					310					315					320
Pro	Gly	Lys	Asn	Ala	Lys	Trp	Glu	Asn	Leu	Glu	Cys	Val	Gln	Lys	Leu
				325					330					335	
Gly	Tyr	Ile	Cys	Lys	Lys	Gly	Asn	Thr	Thr	Leu	Asn	Ser	Phe	Val	Ile
			340					345					350		
Pro	Ser	Glu	Ser	Asp	Val	Pro	Thr	His	Cys	Pro	Ser	Gln	Trp	Trp	Pro
		355					360					365			
Tyr	Ala	Gly	His	Cys	Tyr	Lys	Ile	His	Arg	Asp	Glu	Lys	Lys	Ile	Gln
	370					375					380				
Arg	Asp	Ala	Leu	Thr	Thr	Cys	Arg	Lys	Glu	Gly	Gly	Asp	Leu	Thr	Ser
385					390					395					400
Ile	His	Thr	Ile	Glu	Glu	Leu	Asp	Phe	Ile	Ile	Ser	Gln	Leu	Gly	Tyr
				405					410					415	
Glu	Pro	Asn	Asp	Glu	Leu	Trp	Ile	Gly	Leu	Asn	Asp	Ile	Lys	Ile	Gln
			420					425					430		
Met	Tyr	Phe	Glu	Trp	Ser	Asp	Gly	Thr	Pro	Val	Thr	Phe	Thr	Lys	Trp
		435					440					445			
Leu	Arg	Gly	Glu	Pro	Ser	His	Glu	Asn	Asn	Arg	Gln	Glu	Asp	Cys	Val
	450					455					460				
Val	Met	Lys	Gly	Lys	Asp	Gly	Tyr	Trp	Ala	Asp	Arg	Gly	Cys	Glu	Trp
465					470					475					480
Pro	Leu	Gly	Tyr	Ile	Cys	Lys	Met	Lys	Ser	Arg	Ser	Gln	Gly	Pro	Glu
				485					490					495	
Ile	Val	Glu	Val	Glu	Lys	Gly	Cys	Arg	Lys	Gly	Trp	Lys	Lys	His	His
			500					505					510		

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Phe	Tyr	Cys	Tyr	Met	Ile	Gly	His	Thr	Leu	Ser	Thr	Phe	Ala	Glu	Ala
		515					520					525			
Asn	Gln	Thr	Cys	Asn	Asn	Glu	Asn	Ala	Tyr	Leu	Thr	Thr	Ile	Glu	Asp
	530					535					540				
Arg	Tyr	Glu	Gln	Ala	Phe	Leu	Thr	Ser	Phe	Val	Gly	Leu	Arg	Pro	Glu
545					550					555					560
Lys	Tyr	Phe	Trp	Thr	Gly	Leu	Ser	Asp	Ile	Gln	Thr	Lys	Gly	Thr	Phe
				565					570					575	
Gln	Trp	Thr	Ile	Glu	Glu	Glu	Val	Arg	Phe	Thr	His	Trp	Asn	Ser	Asp
			580					585					590		
Met	Pro	Gly	Arg	Lys	Pro	Gly	Cys	Val	Ala	Met	Arg	Thr	Gly	Ile	Ala
		595					600					605			
Gly	Gly	Leu	Trp	Asp	Val	Leu	Lys	Cys	Asp	Glu	Lys	Ala	Lys	Phe	Val
	610					615					620				
Cys	Lys	His	Trp	Ala	Glu	Gly	Val	Thr	His	Pro	Pro	Lys	Pro	Thr	Thr
625					630					635					640
Thr	Pro	Glu	Pro	Lys	Cys	Pro	Glu	Asp	Trp	Gly	Ala	Ser	Ser	Arg	Thr
				645					650					655	
Ser	Leu	Cys	Phe	Lys	Leu	Tyr	Ala	Lys	Gly	Lys	His	Glu	Lys	Lys	Thr
			660					665					670		
Trp	Phe	Glu	Ser	Arg	Asp	Phe	Cys	Arg	Ala	Leu	Gly	Gly	Asp	Leu	Ala
		675					680					685			
Ser	Ile	Asn	Asn	Lys	Glu	Glu	Gln	Gln	Thr	Ile	Trp	Arg	Leu	Ile	Thr
	690					695					700				
Ala	Ser	Gly	Ser	Tyr	His	Lys	Leu	Phe	Trp	Leu	Gly	Leu	Thr	Tyr	Gly
705					710					715					720
Ser	Pro	Ser	Glu	Gly	Phe	Thr	Trp	Ser	Asp	Gly	Ser	Pro	Val	Ser	Tyr
				725					730					735	
Glu	Asn	Trp	Ala	Tyr	Gly	Glu	Pro	Asn	Asn	Tyr	Gln	Asn	Val	Glu	Tyr
			740					745					750		
Cys	Gly	Glu	Leu	Lys	Gly	Asp	Pro	Thr	Met	Ser	Trp	Asn	Asp	Ile	Asn
		755					760					765			
Cys	Glu	His	Leu	Asn	Asn	Trp	Ile	Cys	Gln	Ile	Gln	Lys	Gly	Gln	Thr
	770					775					780				
Pro	Lys	Pro	Glu	Pro	Thr	Pro	Ala	Pro	Gln	Asp	Asn	Pro	Pro	Val	Thr
					790					795					800
Glu	Asp	Gly	Trp	Val	Ile	Tyr	Lys	Asp	Tyr	Gln	Tyr	Tyr	Phe	Ser	Lys
				805					810					815	
Glu	Lys	Glu	Thr	Met	Asp	Asn	Ala	Arg	Ala	Phe	Cys	Lys	Arg	Asn	Phe
			820					825					830		
Gly	Asp	Leu	Val	Ser	Ile	Gln	Ser	Glu	Ser	Glu	Lys	Lys	Phe	Leu	Trp
		835					840					845			
Lys	Tyr	Val	Asn	Arg	Asn	Asp	Ala	Gln	Ser	Ala	Tyr	Phe	Ile	Gly	Leu
	850					855					860				

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Leu 865	Ile	Ser	Leu	Asp	Lys 870	Lys	Phe	Ala	Trp	Met 875	Asp	Gly	Ser	Lys	Val 880
Asp	Tyr	Val	Ser	Trp 885	Ala	Thr	Gly	Glu	Pro 890	Asn	Phe	Ala	Asn	Glu 895	Asp
Glu	Asn	Cys	Val 900	Thr	Met	Tyr	Ser	Asn 905	Ser	Gly	Phe	Trp	Asn 910	Asp	Ile
Asn	Cys	Gly 915	Tyr	Pro	Asn	Ala	Phe 920	Ile	Cys	Gln	Arg	His 925	Asn	Ser	Ser
Ile 930	Asn	Ala	Thr	Thr	Val	Met 935	Pro	Thr	Met	Pro	Ser 940	Val	Pro	Ser	Gly
Cys 945	Lys	Glu	Gly	Trp	Asn 950	Phe	Tyr	Ser	Asn	Lys 955	Cys	Phe	Lys	Ile	Phe 960
Gly	Phe	Met	Glu	Glu 965	Glu	Arg	Lys	Asn	Trp 970	Gln	Glu	Ala	Arg	Lys 975	Ala
Cys	Ile	Gly 980	Phe	Gly	Gly	Asn	Leu 985	Val	Ser	Ile	Gln	Asn 990	Glu	Lys	Glu
Gln	Ala	Phe 995	Leu	Thr	Tyr	His	Met 1000	Lys	Asp	Ser	Thr	Phe 1005	Ser	Ala	Trp
Thr 1010	Gly	Leu	Asn	Asp	Val	Asn 1015	Ser	Glu	His	Thr	Phe 1020	Leu	Trp	Thr	Asp
Gly 1025	Arg	Gly	Val	His 1030	Tyr	Thr	Asn	Trp	Gly	Lys 1035	Gly	Tyr	Pro	Gly 1040	Gly
Arg	Arg	Ser	Ser	Leu 1045	Ser	Tyr	Glu	Asp	Ala 1050	Asp	Cys	Val	Val	Ile 1055	Ile
Gly	Gly	Ala	Ser 1060	Asn	Glu	Ala	Gly	Lys 1065	Trp	Met	Asp	Asp	Thr 1070	Cys	Asp
Ser	Lys 1075	Arg	Gly	Tyr	Ile	Cys	Gln 1080	Thr	Arg	Ser	Asp 1085	Pro	Ser	Leu	Thr
Asn 1090	Pro	Pro	Ala	Thr	Ile	Gln 1095	Thr	Asp	Gly	Phe	Val 1100	Lys	Tyr	Gly	Lys
Ser 1105	Ser	Tyr	Ser	Leu 1110	Met	Arg	Gln	Lys	Phe	Gln 1115	Trp	His	Glu	Ala	Glu 1120
Thr	Tyr	Cys	Lys	Leu 1125	His	Asn	Ser	Leu	Ile 1130	Ala	Ser	Ile	Leu	Asp 1135	Pro
Tyr	Ser	Asn	Ala 1140	Phe	Ala	Trp	Leu	Gln 1145	Met	Glu	Thr	Ser	Asn 1150	Glu	Arg
Val	Trp	Ile	Ala 1155	Leu	Asn	Ser	Asn 1160	Leu	Thr	Asp	Asn	Gln	Tyr	Thr	Trp
Thr 1170	Asp	Lys	Trp	Arg	Val	Arg 1175	Tyr	Thr	Asn	Trp	Ala 1180	Ala	Asp	Glu	Pro
Lys 1185	Leu	Lys	Ser	Ala	Cys 1190	Val	Tyr	Leu	Asp	Leu 1195	Asp	Gly	Tyr	Trp	Lys 1200
Thr	Ala	His	Cys	Asn 1205	Glu	Ser	Phe	Tyr	Phe 1210	Leu	Cys	Lys	Arg	Ser	Asp 1215

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Glu Ile Pro Ala Thr Glu Pro Pro Gln Leu Pro Gly Arg Cys Pro Glu
 1220 1225 1230
 Ser Asp His Thr Ala Trp Glu Ile Pro Phe His Gly His Cys Tyr Tyr
 1235 1240 1245
 Ile Glu Ser Ser Tyr Thr Arg Asn Trp Gly Gln Ala Ser Leu Glu Cys
 1250 1255 1260
 Leu Arg Met Gly Ser Ser Leu Val Ser Ile Glu Ser Ala Ala Glu Ser
 1265 1270 1275 1280
 Ser Phe Leu Ser Tyr Arg Val Glu Pro Leu Lys Ser Lys Thr Asn Phe
 1285 1290 1295
 Trp Ile Gly Leu Phe Arg Asn Val Glu Gly Thr Trp Leu Trp Ile Asn
 1300 1305 1310
 Asn Ser Pro Val Ser Phe Val Asn Trp Asn Thr Gly Asp Pro Ser Gly
 1315 1320 1325
 Glu Arg Asn Asp Cys Val Ala Leu His Ala Ser Ser Gly Phe Trp Ser
 1330 1335 1340
 Asn Ile His Cys Ser Ser Tyr Lys Gly Tyr Ile Cys Lys Arg Pro Lys
 1345 1350 1355 1360
 Ile Ile Asp Ala Lys Pro Thr His Glu Leu Leu Thr Thr Lys Ala Asp
 1365 1370 1375
 Thr Arg Lys Met Asp Pro Ser Lys Pro Ser Ser Asn Val Ala Gly Val
 1380 1385 1390
 Val Ile Ile Val Ile Leu Leu Ile Leu Thr Gly Ala Gly Leu Ala Ala
 1395 1400 1405
 Tyr Phe Phe Tyr Lys Lys Arg Arg Val His Leu Pro Gln Glu Gly Ala
 1410 1415 1420
 Phe Glu Asn Thr Leu Tyr Phe Asn Ser Gln Ser Ser Pro Gly Thr Ser
 1425 1430 1435 1440
 Asp Met Lys Asp Leu Val Gly Asn Ile Glu Gln Asn Glu His Ser Val
 1445 1450 1455
 Ile
 1457

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
 - (A) DESCRIPTION: murine DEC-205
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: C-terminal
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: murine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Arg	Ser	His	Ile	Arg	Trp	Thr	Gly	Phe	Ser	Ser	Val	Arg	Tyr	Glu	His
1				5					10					15	
Gly	Thr	Asn	Glu	Asp	Glu	Val	Met	Leu	Pro	Ser	Phe	His	Asp		
			20					25					30		

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5477 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:

- (B) CLONE: DEC-205

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATTCGGG	GGCGGGAGCC	GCGTGC GCC	GAGGACCCGG	CCGGAAGGCT	TGCGCCAGCT	60
CAGGATGAGG	ACAGGCTGGG	CGACCCCTCG	CCGCCCCGGC	GGGCTCCTCA	TGCTGCTCTT	120
CTGGTTCTTC	GATCTCGCGG	AGCCCTCTGG	CCGCGCAGCT	AATGACCCCT	TCACCATCGT	180
CCATGGAAAT	ACGGGCAAGT	GCATCAAGCC	AGTGTATGGC	TGGATAGTAG	CAGACGACTG	240
TGATGAAACT	GAGGACAAGT	TATGGAAGTG	GGTGTCCCAG	CATCGGCTCT	TTCATTTGCA	300
CTCCCAAAG	TGCCTTGGCC	TCGATATTAC	CAAATCGGTA	AATGAGCTGA	GAATGTTTCA	360
CTGTGACTCC	AGTGCCATGC	TGTGGTGGAA	ATGTGAGCAC	CACTCTCTGT	ACGGAGCTGC	420
CCGGTACCGG	CTGGCTCTGA	AGGATGGACA	TGGCACAGCA	ATCTCAAATG	CATCTGATGT	480
CTGGAAGAAA	GGAGGCTCAG	AGGAAAGCCT	TTGTGACCAG	CCTTATCATG	AGATCTATAC	540
CAGAGATGGG	AACTCTTATG	GGAGACCTTG	TGAATTTCCA	TTCTTAATTG	ATGGGACCTG	600
GCATCATGAT	TGCATTCTTG	ATGAAGATCA	TAGTGGGCCA	TGGTGTGCCA	CCACCTTAAA	660
TTATGAATAT	GACCGAAAGT	GGGGCATCTG	CTTAAAGCCT	GAAAACGGTT	GTGAAGATAA	720
TTGGGAAAAG	AACGAGCAGT	TTGGAAGTTG	CTACCAATTT	AATACTCAGA	CGGCTCTTTC	780
TTGGAAAGAA	GCTTATGTTT	CATGTCAGAA	TCAAGGAGCT	GATTTACTGA	GCATCAACAG	840
TGCTGCTGAA	TTAACTTACC	TTAAAGATAA	AGAAGGCATT	GCTAAGATTT	TCTGGATTGG	900
TTTAAATCAG	CTATACTCTG	CTAGAGGCTG	GGAATGGTCA	GACCACAAAC	CATTAACTT	960
TCTCAACTGG	GATCCAGACA	GGCCAGTGC	ACCTACTATA	GGTGGCTCCA	GCTGTGCAAG	1020
AATGGATGCT	GAGTCTGGTC	TGTGGCAGAG	CTTTTCCTGT	GAAGCTCAAC	TGCCCTATGT	1080
CTGCAGGAAA	CCATTAAATA	ATACAGTGGA	GTTAACAGAT	GTCTGGACAT	ACTCAGATAC	1140

CCGCTGTGAT	GCAGGCTGGC	TGCCAAATAA	TGGATTTTGC	TATCTGCTGG	TAAATGAAAG	1200
TAATTCCTGG	GATAAGGCAC	ATGCGAAATG	CAAAGCCTTC	AGTAGTGACC	TAATCAGCAT	1260
TCATTCTCTA	GCAGATGTGG	AGGTGGTTGT	CACAAAACCTC	CATAATGAGG	ATATCAAAGA	1320
AGAAGTGTGG	ATAGGCCTTA	AGAACATAAA	CATACCAACT	TTATTTTCAGT	GGTCAGATGG	1380
TACTGAAGTT	ACTCTAACAT	ATTGGGATGA	GAATGAGCCA	AATGTTCCCT	ACAATAAGAC	1440
GCCCAACTGT	GTTTCCTACT	TAGGAGAGCT	AGGTCAGTGG	AAAGTCCAAT	CATGTGAGGA	1500
GAAACTAAAA	TATGTATGCA	AGAGAAAGGG	AGAAAACTG	AATGACGCAA	GTTCTGATAA	1560
GATGTGTCCCT	CCAGATGAGG	GCTGGAAGAG	ACATGGAGAA	ACCTGTTACA	AGATTTATGA	1620
GGATGAGGTC	CCTTTTGGAA	CAAACTGCAA	TCTGACTATC	ACTAGCAGAT	TTGAGCAAGA	1680
ATACCTAAAT	GATTTGATGA	AAAAGTATGA	TAAATCTCTA	AGAAAATACT	TCTGGACTGG	1740
CCTGAGAGAT	GTAGATTCTT	GTGGAGAGTA	TAACTGGGCA	ACTGTTGGTG	GAAGAAGGCG	1800
GGCTGTAACC	TTTTCCAACCT	GGAATTTTCT	TGAGCCAGCT	TCCCCGGGCG	GCTGCGTGGC	1860
TATGTCTACT	GGAAAGTCTG	TTGGAAAGTG	GGAGGTGAAG	GAAGTGCAGAA	GCTTCAAAGC	1920
ACTTTCAATT	TGCAAGAAAA	TGAGTGGACC	CCTTGGGCCT	GAAGAAGCAT	CCCCTAAGCC	1980
TGATGACCCC	TGTCCTGAAG	GCTGGCAGAG	TTTCCCCGCA	AGTCTTTCTT	GTTATAAGGT	2040
ATTCCATGCA	GAAAGAATTG	TAAGAAAGAG	GAAGTGGGAA	GAAGCTGAAC	GATTCTGCCA	2100
AGCCCTTGGA	GCACACCTTT	CTAGCTTCAG	CCATGTGGAT	GAAATAAAGG	AATTTCTTCA	2160
CTTTTTTAACG	GACCAGTTCA	GTGGCCAGCA	TTGGCTGTGG	ATTGGTTTGA	ATAAAAGGAG	2220
CCCAGATTTA	CAAGGATCCT	GGCAATGGAG	TGATCGTACA	CCAGTGTCTA	CTATTATCAT	2280
GCCAAATGAG	TTTCAGCAGG	ATTATGACAT	CAGAGACTGT	GCTGCTGTCA	AGGTATTTCA	2340
TAGGCCATGG	CGAAGAGGCT	GGCATTCTTA	TGATGATAGA	GAATTTATTT	ATTTGAGGCC	2400
TTTTGCTTGT	GATACAAAAC	TTGAATGGGT	GTGCCAAATT	CCAAAAGGCC	GTAATCCAAA	2460
AACACCAGAC	TGGTACAATC	CAGAGCGTGC	TGGAATTCAT	GGACCTCCAC	TTATAATTGA	2520
AGGAAGTGAA	TATTGGTTTG	TTGCTGATCT	TCACCTAAAC	TATGAAGAAG	CCGTCTGTGA	2580
CTGTGCCAGC	AATCACAGCT	TTCTTGCAAC	TATAACATCT	TTTGTGGGAC	TAAAAGCCAT	2640
CAAAAACAAA	ATAGCAAATA	TATCTGGTGA	TGGACAGAAG	TGGTGGATAA	GAATTAGCGA	2700
GTGGCCAATA	GATGATCATT	TTACATACTC	ACGATATCCA	TGGCACCCTG	TTCCTGTGAC	2760
ATTTGGAGAG	GAATGCTTGT	ACATGTCTGC	CAAGACTTGG	CTTATCGACT	TAGGTAAACC	2820
AACAGACTGT	AGTACCAAGT	TGCCCTTCAT	CTGTGAAAAA	TATAATGTTT	CTTCGTTAGA	2880
GAAATACAGC	CCAGATTCTG	CAGCTAAAGT	GCAATGTTCT	GAGCAATGGA	TTCCTTTTCA	2940
GAATAAGTGT	TTTCTAAAGA	TCAAACCCGT	GTCTCTCACA	TTTTCTCAAG	CAAGCGATAC	3000
CTGTCACTCC	TATGGTGGCA	CCCTTCCTTC	AGTGTGAGC	CAGATTGAAC	AAGACTTTAT	3060
TACATCCTTG	CTTCCGGATA	TGGAAGCTAC	TTTATGGATT	GGTTTGCGCT	GGACTGCCTA	3120

TGAAAAGATA	AACAAATGGA	CAGATAACAG	AGAGCTGACG	TACAGTAACT	TTCACCCATT	3180
ATTGGTTAGT	GGGAGGCTGA	GAATACCAGA	AAATTTTTTT	GAGGAAGAGT	CTCGCTACCA	3240
CTGTGCCCTA	ATACTCAACC	TCCAAAAATC	ACCGTTTACT	GGGACGTGGA	ATTTTACATC	3300
CTGCAGTGAA	CGCCACTTTG	TGTCTCTCTG	TCAGAAATAT	TCAGAAAGTTA	AAAGCAGACA	3360
GACGTTGCAG	AATGCTTCAG	AAACTGTAAA	GTATCTAAAT	AATCTGTACA	AAATAATCCC	3420
AAAGACTCTG	ACTTGGCACA	GTGCTAAAAG	GGAGTGTCTG	AAAAGTAACA	TGCAGCTGGT	3480
GAGCATCACG	GACCCTTACC	AGCAGGCATT	CCTCAGTGTG	CAGGCGCTCC	TTCACAACTC	3540
TTCCTTATGG	ATCGGACTCT	TCAGTCAAGA	TGATGAACTC	AACTTTGGTT	GGTCAGATGG	3600
GAAACGTCTT	CATTTTAGTC	GCTGGGCTGA	AACTAATGGG	CAACTCGAAG	ACTGTGTAGT	3660
ATTAGACACT	GATGGATTCT	GGAAAACAGT	TGATTGCAAT	GACAATCAAC	CAGGTGCTAT	3720
TTGCTACTAT	CCAGGAAATG	AGACTGAAAA	AGAGGTCAAA	CCAGTTGACA	GTGTTAAATG	3780
TCCATCTCCT	GTTCTAAATA	CTCCGTGGAT	ACCATTTTCAG	AACTGTTGCT	ACAATTTTCAT	3840
AATAACAAAG	AATAGGCATA	TGGCAACAAC	ACAGGATGAA	GTTCACTACTA	AATGCCAGAA	3900
ACTGAATCCA	AAATCACATA	TTCTGAGTAT	TCGAGATGAA	AAGGAGAATA	ACTTTGTTCT	3960
TGAGCAACTG	CTGTACTTCA	ATTATATGGC	TTCATGGGTC	ATGTTAGGAA	TAAGTTATAG	4020
AAATAATTCT	CTTATGTGGT	TTGATAAGAC	CCCACTGTCA	TATACACATT	GGAGAGCAGG	4080
AAGACCAACT	ATAAAAAATG	AGAGGTTTTT	GGCTGGTTTA	AGTACTGACG	GCTTCTGGGA	4140
TATTCAAACC	TTTAAAGTTA	TTGAAGAAGC	AGTTTATTTT	CACCAGCACA	GCATTCTTGC	4200
TTGTAAAATT	GAAATGGTTG	ACTACAAAGA	AGAATATAAT	ACTACACTGC	CACAGTTTAT	4260
GCCATATGAA	GATGGTATTT	ACAGTGTTAT	TCAAAAAAAG	GTAACATGGT	ATGAAGCATT	4320
AAACATGTGT	TCTCAAAGTG	GAGGTCACCT	GGCAAGCGTT	CACAACCAAA	ATGGCCAGCT	4380
CTTTCTGGAA	GATATTGTAA	AACGTGATGG	ATTTCCACTA	TGGGTTGGGC	TCTCAAGTCA	4440
TGATGGAAGT	GAATCAAGTT	TTGAATGGTC	TGATGGTAGT	ACATTTGACT	ATATCCCATG	4500
GAAAGGCCAA	ACATCTCCTG	GAAATTGTGT	TCTCTTGGAT	CCAAAAGGAA	CTTGGAACA	4560
TGAAAAATGC	AACTCTGTTA	AGGATGGTGC	TATTTGTTAT	AAACCTACAA	AAGCTAAAAA	4620
GCTGTCCCGT	CTTACATATT	CATCAAGATG	TCCAGCAGCA	AAAGAGAATG	GGTCACGGTG	4680
GATCCAGTAC	AAGGGTCACT	GTTACAAGTC	TGATCAGGCA	TTGCACAGTT	TTTCAGAGGC	4740
CAAAAAATTG	TGTTCAAAAC	ATGATCACTC	TGCAACTATC	GTTTCCATAA	AAGATGAAGA	4800
TGAGAATAAA	TTTGTGAGCA	GACTGATGAG	GGAAAATAAT	AACATTACCA	TGAGAGTTTG	4860
GCTTGGATTA	TCTCAACATT	CTGTTGACCA	GTCTTGGAGT	TGGTTAGATG	GATCAGAAGT	4920
GACATTTGTC	AAATGGGAAA	ATAAAAGTAA	GAGTGGTGTG	GGAAGATGTA	GCATGTTGAT	4980
AGCTTCAAAT	GAAACTTGGA	AAAAAGTTGA	ATGTGAACAT	GGTTTTGGAA	GAGTTGTCTG	5040
CAAAGTGCCT	CTGGGCCCTG	ATTACACAGC	AATAGCTATC	ATAGTTGCCA	CACTAAGTAT	5100

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CTTAGTTCTC ATGGGCGGAC TGATTTGGTT CCTCTTCCAA AGGCACCGTT TGCACCTGGC      5160
GGGTTTCTCA TCAGTTCGAT ATGCACAAGG AGTGAATGAA GATGAGATTA TGCTTCCTTC      5220
TTTCCATGAC TAAATTCTTC TAAAAGTTTT CTAATTTGCA CTAATGTGTT ATGAGAAATT      5280
AGTCACTTAA AATGTCCAGT GTCAGTATTT ACTCTGCTCC AAAGTAGAAC TCTTAAATAC      5340
TTTTTCAGTT GTTTAGATCT AGGCATGTGC TGGTATCCAC AGTTAATTCC CTGCTAAATG      5400
CCATGTTTAT CACCCTAATT AATAGAATGG AGGGGACTCC AAAGCTGGAA CTGAAGTCAA      5460
ATTGTTTGAC AGTAATA                                                    5477

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(2) INFORMATION FOR SEQ ID NO:8:

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(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 1825 amino acids
    (B) TYPE: amino acid
    (C) STRANDEDNESS: not relevant
    (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vii) IMMEDIATE SOURCE:
    (B) CLONE: DEC-205

Asn Ser Gly Gly Gly Ser Arg Val Arg Pro Arg Thr Arg Pro Glu Gly
 1              5              10              15

Leu Arg Gln Leu Arg Met Arg Thr Gly Trp Ala Thr Pro Arg Arg Pro
 20              25              30

Ala Gly Leu Leu Met Leu Leu Phe Trp Phe Phe Asp Leu Ala Glu Pro
 35              40              45

Ser Gly Arg Ala Ala Asn Asp Pro Phe Thr Ile Val His Gly Asn Thr
 50              55              60

Gly Lys Cys Ile Lys Pro Val Tyr Gly Trp Ile Val Ala Asp Asp Cys
 65              70              75              80

Asp Glu Thr Glu Asp Lys Leu Trp Lys Trp Val Ser Gln His Arg Leu
 85              90              95

Phe His Leu His Ser Gln Lys Cys Leu Gly Leu Asp Ile Thr Lys Ser
 100             105             110

Val Asn Glu Leu Arg Met Phe Ser Cys Asp Ser Ser Ala Met Leu Trp
 115             120             125

Trp Lys Cys Glu His His Ser Leu Tyr Gly Ala Ala Arg Tyr Arg Leu
 130             135             140

Ala Leu Lys Asp Gly His Gly Thr Ala Ile Ser Asn Ala Ser Asp Val
 145             150             155             160

Trp Lys Lys Gly Gly Ser Glu Glu Ser Leu Cys Asp Gln Pro Tyr His
 165             170             175

Glu Ile Tyr Thr Arg Asp Gly Asn Ser Tyr Gly Arg Pro Cys Glu Phe

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			180					185					190			
Pro	Phe	Leu	Ile	Asp	Gly	Thr	Trp	His	His	Asp	Cys	Ile	Leu	Asp	Glu	
		195					200					205				
Asp	His	Ser	Gly	Pro	Trp	Cys	Ala	Thr	Thr	Leu	Asn	Tyr	Glu	Tyr	Asp	
	210					215					220					
Arg	Lys	Trp	Gly	Ile	Cys	Leu	Lys	Pro	Glu	Asn	Gly	Cys	Glu	Asp	Asn	
225					230					235					240	
Trp	Glu	Lys	Asn	Glu	Gln	Phe	Gly	Ser	Cys	Tyr	Gln	Phe	Asn	Thr	Gln	
				245					250					255		
Thr	Ala	Leu	Ser	Trp	Lys	Glu	Ala	Tyr	Val	Ser	Cys	Gln	Asn	Gln	Gly	
			260					265					270			
Ala	Asp	Leu	Leu	Ser	Ile	Asn	Ser	Ala	Ala	Glu	Leu	Thr	Tyr	Leu	Lys	
		275					280					285				
Asp	Lys	Glu	Gly	Ile	Ala	Lys	Ile	Phe	Trp	Ile	Gly	Leu	Asn	Gln	Leu	
	290					295					300					
Tyr	Ser	Ala	Arg	Gly	Trp	Glu	Trp	Ser	Asp	His	Lys	Pro	Leu	Asn	Phe	
305					310					315					320	
Leu	Asn	Trp	Asp	Pro	Asp	Arg	Pro	Ser	Ala	Pro	Thr	Ile	Gly	Gly	Ser	
				325					330					335		
Ser	Cys	Ala	Arg	Met	Asp	Ala	Glu	Ser	Gly	Leu	Trp	Gln	Ser	Phe	Ser	
			340					345					350			
Cys	Glu	Ala	Gln	Leu	Pro	Tyr	Val	Cys	Arg	Lys	Pro	Leu	Asn	Asn	Thr	
		355					360					365				
Val	Glu	Leu	Thr	Asp	Val	Trp	Thr	Tyr	Ser	Asp	Thr	Arg	Cys	Asp	Ala	
	370					375					380					
Gly	Trp	Leu	Pro	Asn	Asn	Gly	Phe	Cys	Tyr	Leu	Leu	Val	Asn	Glu	Ser	
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Asn	Ser	Trp	Asp	Lys	Ala	His	Ala	Lys	Cys	Lys	Ala	Phe	Ser	Ser	Asp	
				405					410					415		
Leu	Ile	Ser	Ile	His	Ser	Leu	Ala	Asp	Val	Glu	Val	Val	Val	Thr	Lys	
			420					425					430			
Leu	His	Asn	Glu	Asp	Ile	Lys	Glu	Glu	Val	Trp	Ile	Gly	Leu	Lys	Asn	
		435					440					445				
Ile	Asn	Ile	Pro	Thr	Leu	Phe	Gln	Trp	Ser	Asp	Gly	Thr	Glu	Val	Thr	
	450					455					460					
Leu	Thr	Tyr	Trp	Asp	Glu	Asn	Glu	Pro	Asn	Val	Pro	Tyr	Asn	Lys	Thr	
465					470					475					480	
Pro	Asn	Cys	Val	Ser	Tyr	Leu	Gly	Glu	Leu	Gly	Gln	Trp	Lys	Val	Gln	
				485					490					495		
Ser	Cys	Glu	Glu	Lys	Leu	Lys	Tyr	Val	Cys	Lys	Arg	Lys	Gly	Glu	Lys	
			500					505					510			
Leu	Asn	Asp	Ala	Ser	Ser	Asp	Lys	Met	Cys	Pro	Pro	Asp	Glu	Gly	Trp	
		515</														

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Lys	Arg	His	Gly	Glu	Thr	Cys	Tyr	Lys	Ile	Tyr	Glu	Asp	Glu	Val	Pro
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Phe	Gly	Thr	Asn	Cys	Asn	Leu	Thr	Ile	Thr	Ser	Arg	Phe	Glu	Gln	Glu
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Tyr	Leu	Asn	Asp	Leu	Met	Lys	Lys	Tyr	Asp	Lys	Ser	Leu	Arg	Lys	Tyr
				565					570					575	
Phe	Trp	Thr	Gly	Leu	Arg	Asp	Val	Asp	Ser	Cys	Gly	Glu	Tyr	Asn	Trp
			580					585					590		
Ala	Thr	Val	Gly	Gly	Arg	Arg	Arg	Ala	Val	Thr	Phe	Ser	Asn	Trp	Asn
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Phe	Leu	Glu	Pro	Ala	Ser	Pro	Gly	Gly	Cys	Val	Ala	Met	Ser	Thr	Gly
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Lys	Ser	Val	Gly	Lys	Trp	Glu	Val	Lys	Asp	Cys	Arg	Ser	Phe	Lys	Ala
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Leu	Ser	Ile	Cys	Lys	Lys	Met	Ser	Gly	Pro	Leu	Gly	Pro	Glu	Glu	Ala
				645					650					655	
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His	Leu	Ser	Ser	Phe	Ser	His	Val	Asp	Glu	Ile	Lys	Glu	Phe	Leu	His
705					710					715					720
Phe	Leu	Thr	Asp	Gln	Phe	Ser	Gly	Gln	His	Trp	Leu	Trp	Ile	Gly	Leu
				725					730					735	
Asn	Lys	Arg	Ser	Pro	Asp	Leu	Gln	Gly	Ser	Trp	Gln	Trp	Ser	Asp	Arg
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Thr	Pro	Val	Ser	Thr	Ile	Ile	Met	Pro	Asn	Glu	Phe	Gln	Gln	Asp	Tyr
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Asp	Ile	Arg	Asp	Cys	Ala	Ala	Val	Lys	Val	Phe	His	Arg	Pro	Trp	Arg
	770					775					780				
Arg	Gly	Trp	His	Phe	Tyr	Asp	Asp	Arg	Glu	Phe	Ile	Tyr	Leu	Arg	Pro
785					790					795					800
Phe	Ala	Cys	Asp	Thr	Lys	Leu	Glu	Trp	Val	Cys	Gln	Ile	Pro	Lys	Gly
				805					810					815	
Arg	Thr	Pro	Lys	Thr	Pro	Asp	Trp	Tyr	Asn	Pro	Glu	Arg	Ala	Gly	Ile
			820					825					830		
His	Gly	Pro	Pro	Leu	Ile	Ile	Glu	Gly	Ser	Glu	Tyr	Trp	Phe	Val	Ala
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Asp	Leu	His	Leu	Asn	Tyr	Glu	Glu	Ala	Val	Leu	Tyr	Cys	Ala	Ser	Asn
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His	Ser	Phe	Leu	Ala	Thr	Ile	Thr	Ser	Phe	Val	Gly	Leu	Lys	Ala	Ile
865					870					875					880

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Lys Asn Lys Ile Ala Asn Ile Ser Gly Asp Gly Gln Lys Trp Trp Ile
 885 890 895
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 900 905 910
 Pro Trp His Arg Phe Pro Val Thr Phe Gly Glu Glu Cys Leu Tyr Met
 915 920 925
 Ser Ala Lys Thr Trp Leu Ile Asp Leu Gly Lys Pro Thr Asp Cys Ser
 930 935 940
 Thr Lys Leu Pro Phe Ile Cys Glu Lys Tyr Asn Val Ser Ser Leu Glu
 945 950 955 960
 Lys Tyr Ser Pro Asp Ser Ala Ala Lys Val Gln Cys Ser Glu Gln Trp
 965 970 975
 Ile Pro Phe Gln Asn Lys Cys Phe Leu Lys Ile Lys Pro Val Ser Leu
 980 985 990
 Thr Phe Ser Gln Ala Ser Asp Thr Cys His Ser Tyr Gly Gly Thr Leu
 995 1000 1005
 Pro Ser Val Leu Ser Gln Ile Glu Gln Asp Phe Ile Thr Ser Leu Leu
 1010 1015 1020
 Pro Asp Met Glu Ala Thr Leu Trp Ile Gly Leu Arg Trp Thr Ala Tyr
 1025 1030 1035 1040
 Glu Lys Ile Asn Lys Trp Thr Asp Asn Arg Glu Leu Thr Tyr Ser Asn
 1045 1050 1055
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 Phe Glu Glu Glu Ser Arg Tyr His Cys Ala Leu Ile Leu Asn Leu Gln
 1075 1080 1085
 Lys Ser Pro Phe Thr Gly Thr Trp Asn Phe Thr Ser Cys Ser Glu Arg
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 His Phe Val Ser Leu Cys Gln Lys Tyr Ser Glu Val Lys Ser Arg Gln
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 Thr Leu Gln Asn Ala Ser Glu Thr Val Lys Tyr Leu Asn Asn Leu Tyr
 1125 1130 1135
 Lys Ile Ile Pro Lys Thr Leu Thr Trp His Ser Ala Lys Arg Glu Cys
 1140 1145 1150
 Leu Lys Ser Asn Met Gln Leu Val Ser Ile Thr Asp Pro Tyr Gln Gln
 1155 1160 1165
 Ala Phe Leu Ser Val Gln Ala Leu Leu His Asn Ser Ser Leu Trp Ile
 1170 1175 1180
 Gly Leu Phe Ser Gln Asp Asp Glu Leu Asn Phe Gly Trp Ser Asp Gly
 1185 1190 1195 1200
 Lys Arg Leu His Phe Ser Arg Trp Ala Glu Thr Asn Gly Gln Leu Glu
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 Asp Cys Val Val Leu Asp Thr Asp Gly Phe Trp Lys Thr Val Asp Cys
 1220 1225 1230

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Asn Asp Asn Gln Pro Gly Ala Ile Cys Tyr Tyr Pro Gly Asn Glu Thr
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 Glu Lys Glu Val Lys Pro Val Asp Ser Val Lys Cys Pro Ser Pro Val
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 Leu Asn Thr Pro Trp Ile Pro Phe Gln Asn Cys Cys Tyr Asn Phe Ile
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 1285 1290 1295
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 1315 1320 1325
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 1330 1335 1340
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 Arg Pro Thr Ile Lys Asn Glu Arg Phe Leu Ala Gly Leu Ser Thr Asp
 1365 1370 1375
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 1380 1385 1390
 Phe His Gln His Ser Ile Leu Ala Cys Lys Ile Glu Met Val Asp Tyr
 1395 1400 1405
 Lys Glu Glu Tyr Asn Thr Thr Leu Pro Gln Phe Met Pro Tyr Glu Asp
 1410 1415 1420
 Gly Ile Tyr Ser Val Ile Gln Lys Lys Val Thr Trp Tyr Glu Ala Leu
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 Asn Met Cys Ser Gln Ser Gly Gly His Leu Ala Ser Val His Asn Gln
 1445 1450 1455
 Asn Gly Gln Leu Phe Leu Glu Asp Ile Val Lys Arg Asp Gly Phe Pro
 1460 1465 1470
 Leu Trp Val Gly Leu Ser Ser His Asp Gly Ser Glu Ser Ser Phe Glu
 1475 1480 1485
 Trp Ser Asp Gly Ser Thr Phe Asp Tyr Ile Pro Trp Lys Gly Gln Thr
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 Ser Pro Gly Asn Cys Val Leu Leu Asp Pro Lys Gly Thr Trp Lys His
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 Glu Lys Cys Asn Ser Val Lys Asp Gly Ala Ile Cys Tyr Lys Pro Thr
 1525 1530 1535
 Lys Ala Lys Lys Leu Ser Arg Leu Thr Tyr Ser Ser Arg Cys Pro Ala
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 Ala Lys Glu Asn Gly Ser Arg Trp Ile Gln Tyr Lys Gly His Cys Tyr
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 Lys Ser Asp Gln Ala Leu His Ser Phe Ser Glu Ala Lys Lys Leu Cys
 1570 1575 1580

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Ser Lys His Asp His Ser Ala Thr Ile Val Ser Ile Lys Asp Glu Asp
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 His Ser Xaa Phe Pro Ala Lys Cys His Val Tyr His Pro Asn Xaa Xaa
 1795 1800 1805
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 1810 1815 1820
 Asn
 1825

WHAT IS CLAIMED IS:

- 1 1. A method for identifying a ligand for DEC, wherein DEC is an integral
2 membrane protein expressed by dendritic cells, thymic epithelial cells, lung
3 epithelial cells, small intestine epithelial cells, and brain capillaries, having an
4 apparent molecular weight of 205 kDa by polyacrylamide gel electrophoresis, and
5 comprising ten lectin domains, a transmembrane domain, and a short cytoplasmic
6 tail containing a coated pit localization consensus sequence, which method
7 comprises:
 - 8 a) contacting a protein comprising at least one DEC lectin domain with
9 a candidate ligand; and
 - 10 b) detecting binding of the candidate ligand with the DEC lectin
11 domain;
- 12 wherein detection of binding of the candidate ligand and the DEC lectin domain
13 indicates that the ligand candidate is a ligand for DEC.
- 1 2. The method according to claim 1, wherein the ligand is a saccharide.
- 1 3. The method according to claim 1, wherein the protein comprising at least
2 one DEC lectin domain is expressed by cells as an integral membrane protein, and
3 the candidate ligand is labeled, such that binding of the candidate ligand with the
4 DEC lectin domain is detected by detecting association of the label with the cells.
- 1 4. The method according to claim 1, wherein the protein comprising at least
2 one DEC lectin domain is solubilized, and the candidate ligand is irreversibly
3 associated with a solid phase support, such that binding of the candidate ligand
4 with the DEC lectin domain is detected by detecting binding of the protein with the
5 solid phase support.
- 1 5. The method according to claim 1, wherein the protein comprising at least
2 one DEC lectin domain is irreversibly associated with a solid phase support, and

3 the candidate ligand is labeled, such that binding of the candidate ligand with the
4 DEC lectin domain is detected by detecting association of label with the solid
5 phase support.

1 6. The method according to Claim 1 wherein the protein comprising at least
2 one DEC lectin domain is a truncated DEC protein.

1 7. The method according to Claim 1 wherein the protein comprising at least
2 one DEC lectin domain is a full length DEC protein.

1 8. Human DEC-205, wherein the human DEC-205 is an integral membrane
2 protein expressed by dendritic cells, having an apparent molecular weight of 205
3 kDa by polyacrylamide gel electrophoresis, comprising ten lectin domains, a
4 transmembrane domain, and a short cytoplasmic tail containing a coated pit
5 localization consensus sequence, having a carboxyl-terminal sequence
6 RHRLHLAGFSSVRYAQGVNEDEIMLPSFHD (SEQ ID NO:1), and characterized
7 by binding to a rabbit polyclonal antibody raised against full length murine DEC-
8 205, but not reacting with monoclonal antibody NLDC-145.

1 9. The human DEC-205 of claim 8 having an amino acid sequence as depicted
2 in SEQ ID NO:8.

1 10. A purified nucleic acid encoding at least a portion of human DEC-205 of
2 claim 8, which nucleic acid is characterized by having at least fifteen base pairs.

1 11. The nucleic acid of claim 9 which encodes a lectin binding domain.

1 12. The nucleic acid of claim 10, selected from the group consisting of:
2 a) a nucleic acid having a sequence corresponding to the sequence
3 depicted in SEQ ID NO:7;
4 b) an allelic variant of the nucleic acid of (a);

- 5 c) a nucleic acid encoding a polypeptide having the amino acid
6 sequence depicted in SEQ ID NO:8 up to amino acid 1743; and
7 d) an allelic variant of the nucleic acid of (c).

1 13. A purified nucleic acid encoding at least a portion of a DEC protein,
2 wherein the DEC protein is an integral membrane protein expressed by dendritic
3 cells, having an apparent molecular weight of 205 kDa by polyacrylamide gel
4 electrophoresis, and comprising ten lectin domains, a transmembrane domain, and a
5 short cytoplasmic tail containing a coated pit localization consensus sequence,
6 which nucleic acid comprises at least fifteen base pairs.

1 14. The nucleic acid of claim 13 which encodes a human DEC protein.

1 15. The nucleic acid of claim 13 which encodes a murine DEC protein.

1 16. An expression vector comprising the nucleic acid of any one of claims 10 to
2 15, wherein the nucleic acid is a DNA molecule encoding at least a lectin domain
3 of DEC, operatively associated with an expression control sequence.

1 17. A recombinant host cell comprising the expression vector of claim 16.

1 18. The recombinant host cell of claim 17 which is a mammalian cell selected
2 from the group consisting of a Chinese hamster ovary cell, an African Green
3 Monkey COS cell, a Madin-Darby canine kidney cell, and an NIH-3T3 fibroblast
4 cell.

1 19. The recombinant host cell of claim 18, wherein the DNA molecule encodes
2 a full length DEC protein.

1 20. The recombinant host cell of claim 18, wherein the DNA molecule encodes
2 a human DEC protein.

1 21. An antibody reactive with a human DEC-205 protein, which human DEC-
2 205 is an integral membrane protein expressed by dendritic cells, having an
3 apparent molecular weight of 205 kDa by polyacrylamide gel electrophoresis,
4 comprising ten lectin domains, a transmembrane domain, and a short cytoplasmic
5 tail containing a coated pit localization consensus sequence, and characterized by
6 binding to a rabbit polyclonal antibody raised against full length murine DEC-205,
7 but not reacting with monoclonal antibody NLDC-145.

1 22. The antibody of claim 21, wherein human DEC-205 is characterized by a
2 property selected from the group consisting of:
3 a) having a carboxyl-terminal sequence
4 RHRLHLAGFSSVRYAQGVNEDEIMLPSFHD (SEQ ID NO:1);
5 b) having an amino acid sequence as depicted in SEQ ID NO:8 up to
6 amino acid 1743; and
7 c) being encoded by a DNA molecule having a nucleotide sequence as
8 depicted in SEQ ID NO:7.

1 23. The antibody of claim 21 or 22 which is a monoclonal antibody.

1 24. The antibody of claim 21 or 22 which is a polyclonal antibody.

1 25. A pharmaceutical composition comprising a molecule targeted to a tissue
2 selected from the group consisting of pulmonary circulation, intestinal circulation,
3 pulmonary airways, lumen of the small intestine, dendritic cells in the skin and T
4 cell areas of lymphoid organs, thymus, and brain, which molecule is conjugated to
5 a DEC-ligand, which DEC-ligand is selected from the group consisting of a
6 carbohydrate that binds DEC and an anti-DEC antibody, and a pharmaceutically
7 acceptable carrier.

1 26. The pharmaceutical composition of claim 25, wherein the molecule is
2 selected from the group consisting of an anti-cancer drug, an anti-viral drug, an
3 antibiotic, an anti-parasitic drug, and an anti-inflammatory drug.

1 27. Use of a DEC-ligand, which DEC-ligand is selected from the group
2 consisting of a carbohydrate that binds DEC and an anti-DEC antibody, in the
3 manufacture of a molecule targeted to a tissue selected from the group consisting of
4 pulmonary circulation, intestinal circulation, pulmonary airways, lumen of the small
5 intestine, dendritic cells in the skin and T cell areas of lymphoid organs, thymus,
6 and brain.

1 28. A recombinant vector for introduction of a gene into cells selected from the
2 group consisting of dendritic cells, thymic epithelial cells, lung epithelial cells,
3 small intestine epithelial cells, and brain capillary cells comprising a DNA vector
4 conjugated to a DEC-ligand, wherein the DEC-ligand is selected from the group
5 consisting of a carbohydrate that binds DEC and an anti-DEC antibody.

1 29. The recombinant vector of claim 28 wherein the DNA vector is selected
2 from the group consisting of a viral vector, a liposome vector, and a naked DNA
3 vector.

1 30. A vaccine comprising an antigen from a pathogen conjugated to a DEC-
2 ligand, wherein the DEC-ligand is selected from the group consisting of a
3 carbohydrate that binds DEC and an anti-DEC antibody, and an immune stimulator.

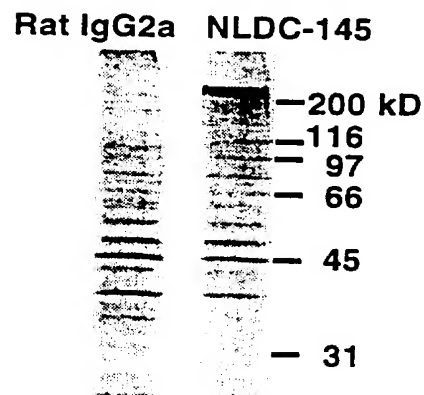
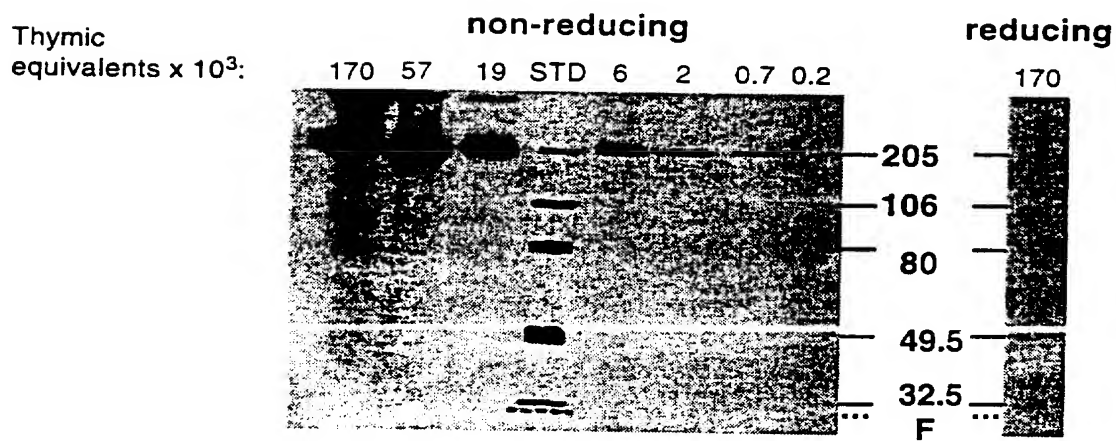
1 31. The vaccine of claim 30, wherein the pathogen is selected from the group
2 consisting of a virus, a bacterium, a parasite, and a tumor.

1 32. The vaccine of claim 30, wherein the immune stimulator is selected from
2 the group consisting of a cytokine, a lymphokine, and an adjuvant.

1 33. A composition to induce immune suppression comprising an autoantigen or
2 an allergen conjugated to a DEC-ligand, wherein the DEC ligand is selected from
3 the group consisting of a carbohydrate that binds DEC and an anti-DEC antibody,
4 with the proviso that the composition lack immune stimulatory agents.

1 34. The composition of claim 33, wherein the autoantigen is selected from the
2 group consisting of myelin basic protein, collagen or a fragment thereof, DNA, a
3 nuclear protein, a nucleolar protein, a mitochondrial protein, and a pancreatic β -cell
4 protein.

Figure 1

A**Immunoprecipitation****B****Western Blot**

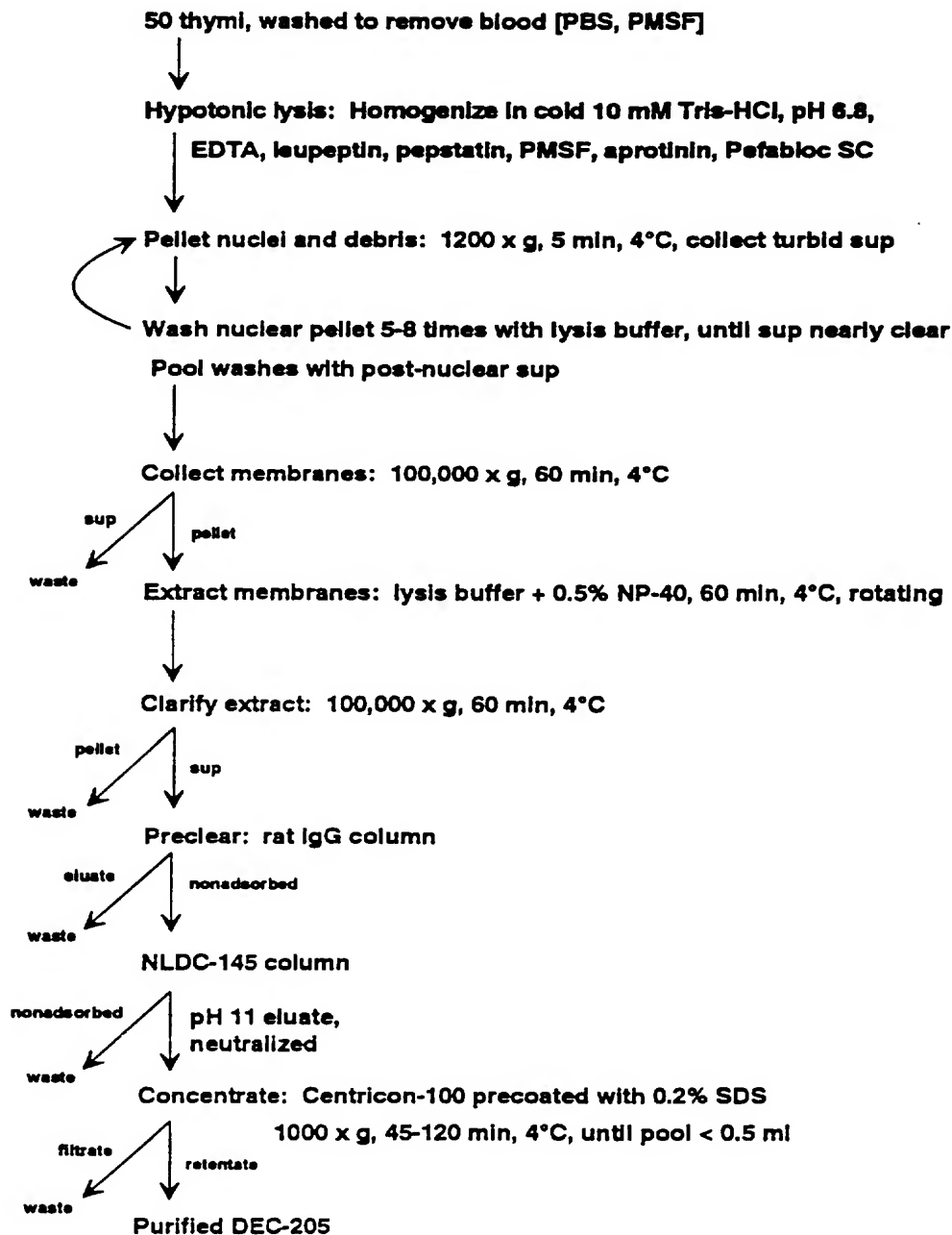
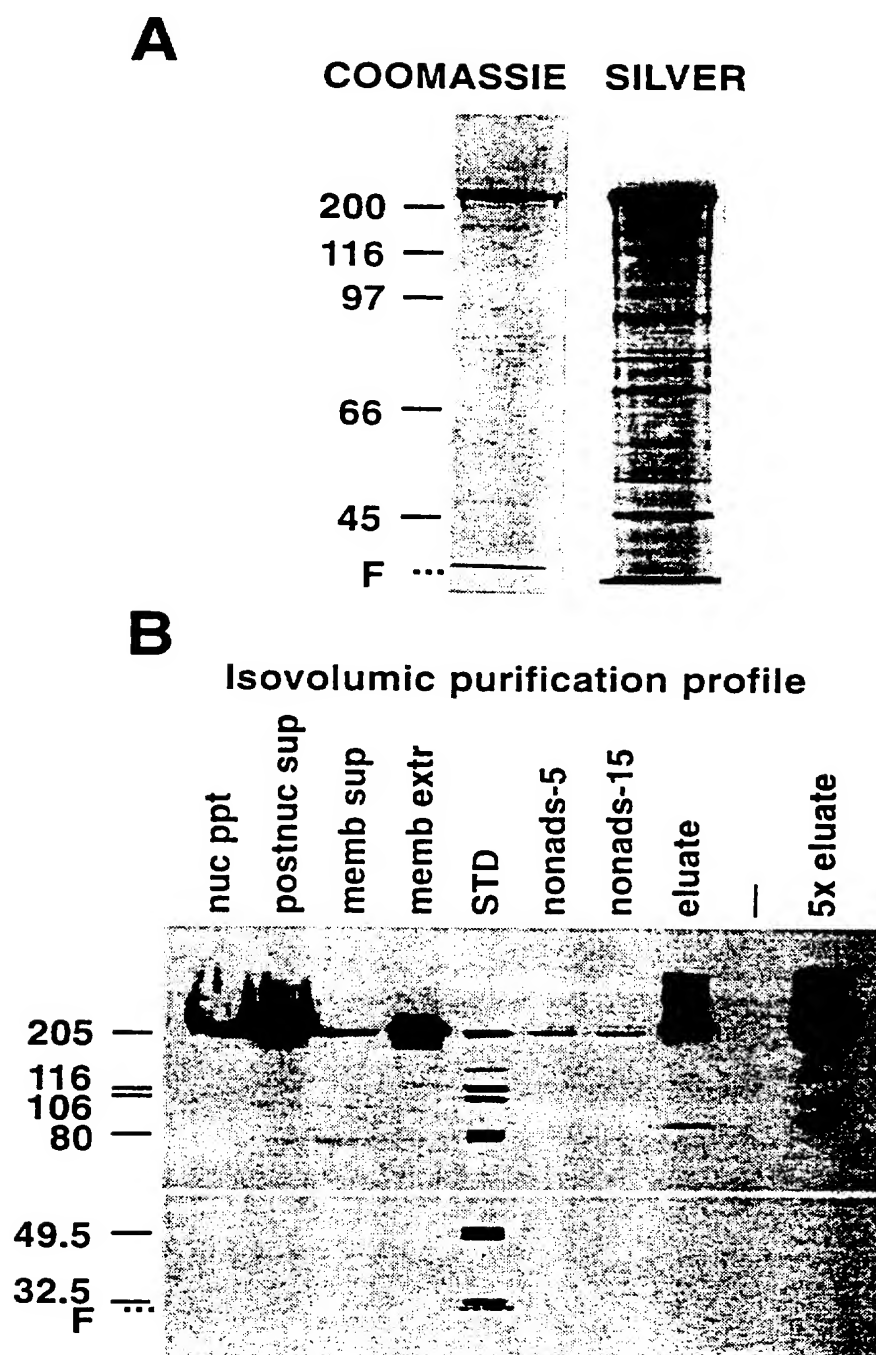
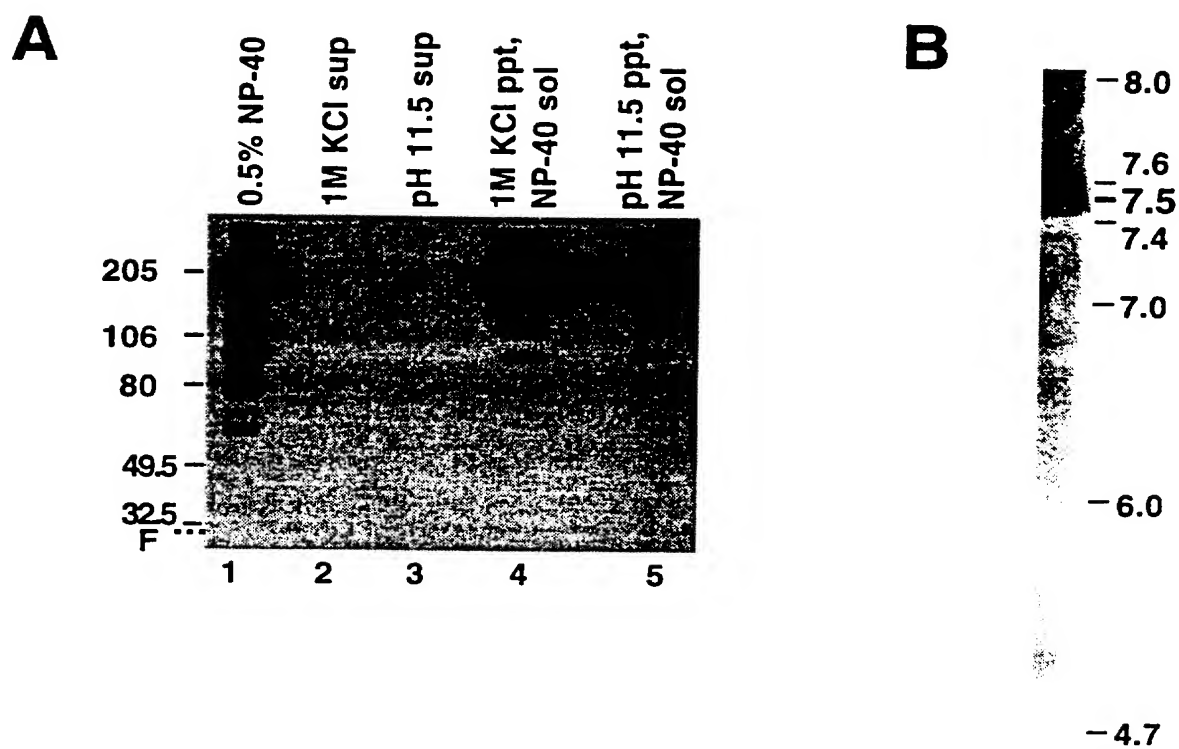
2/26
Figure 2

Figure 3



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Figure 4

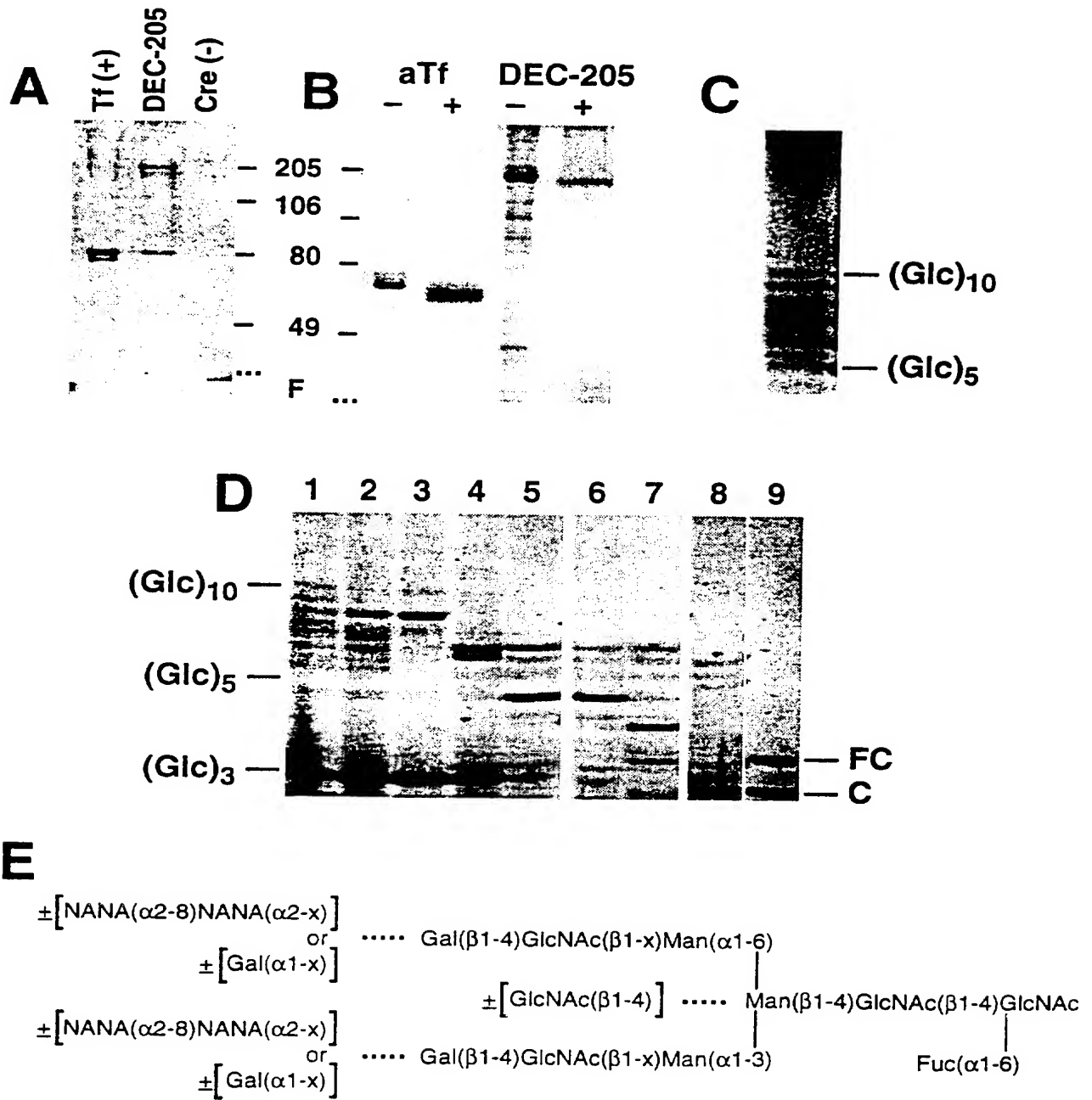


Figure 6

A

1 5 10 15 20 25
S E S S G N D P F T I V H E N T G K C I Q P L F D

B

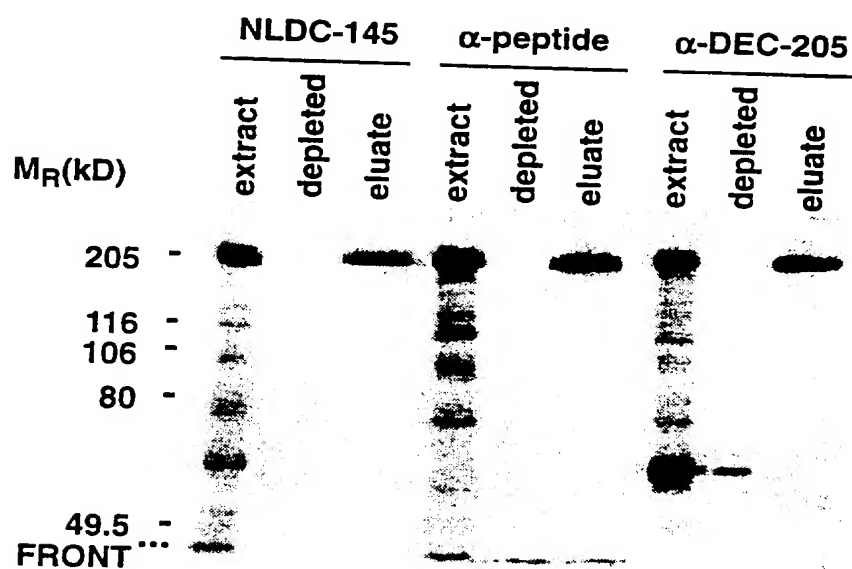
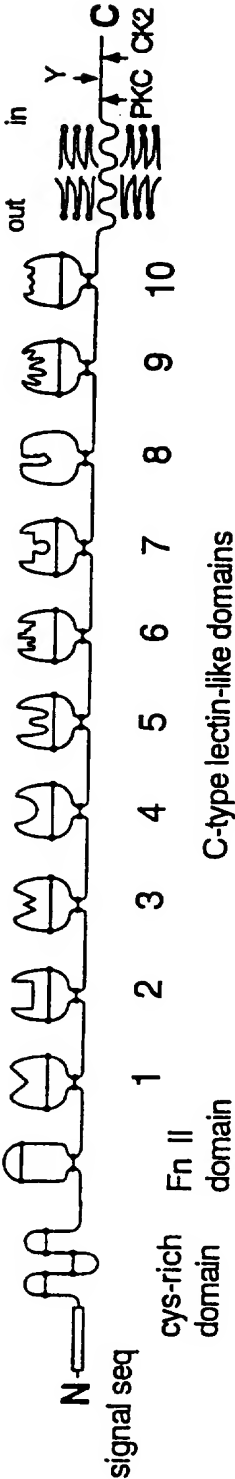


Figure 7



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Figure 8A

[illegible]

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Figure 8B

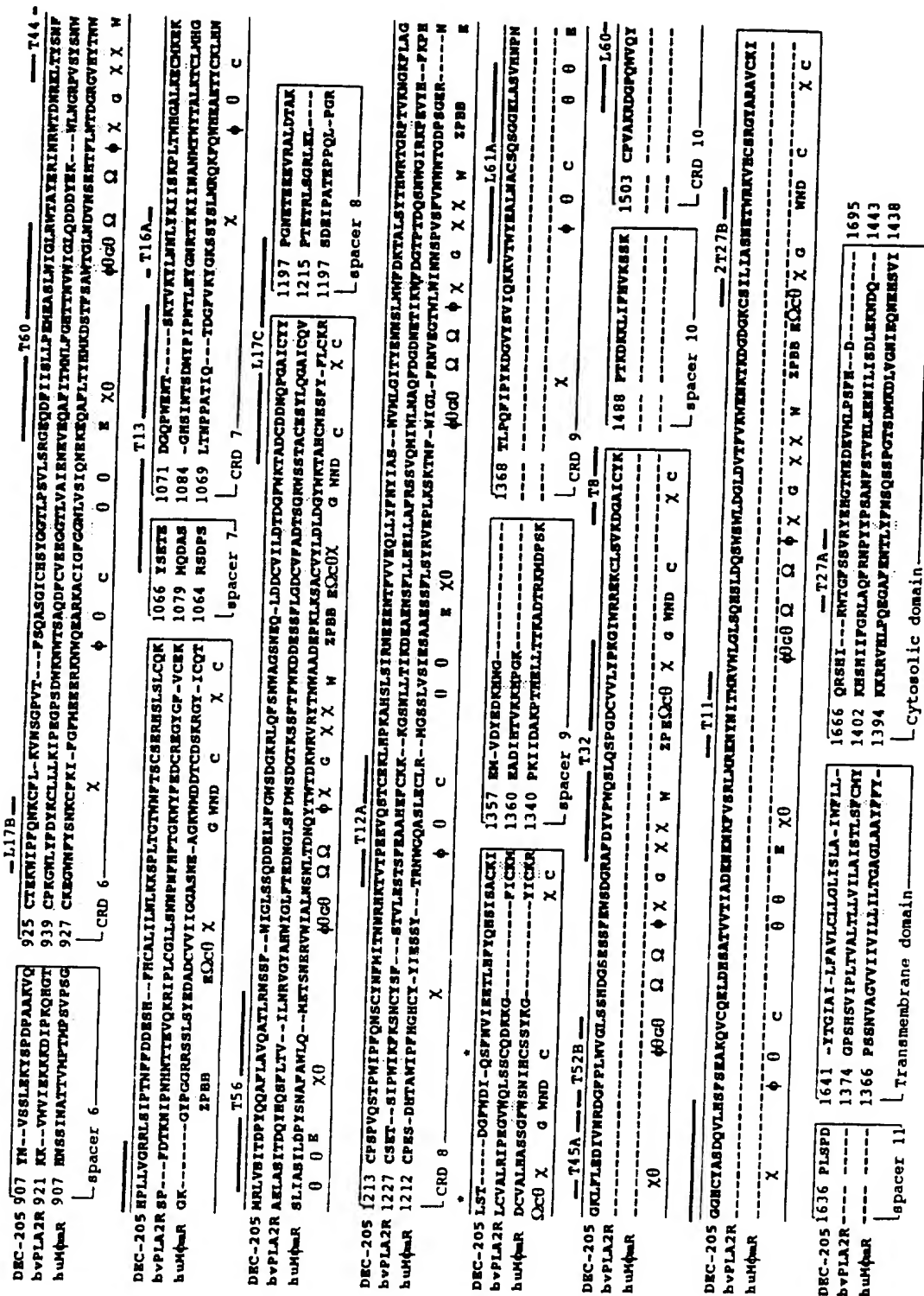


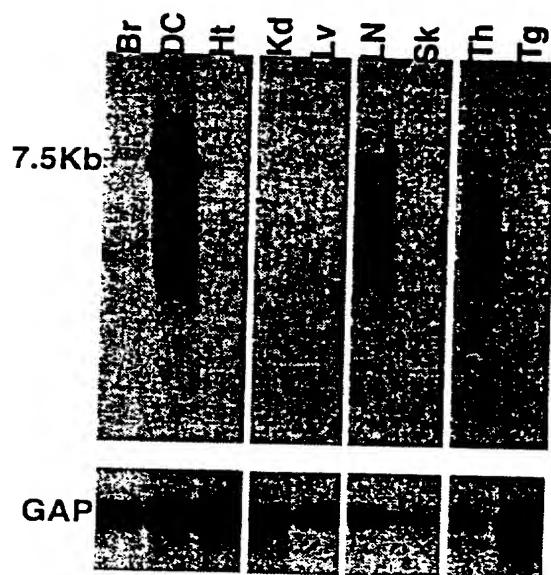
Figure 9

Human

R H R L H L A G F S S V R Y A Q G V N E D E I M L P S F H D
R S H I R W T G F S S V R Y E H G T N E D E V M L P S F H D

Mouse

Figure 10



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Figure 11

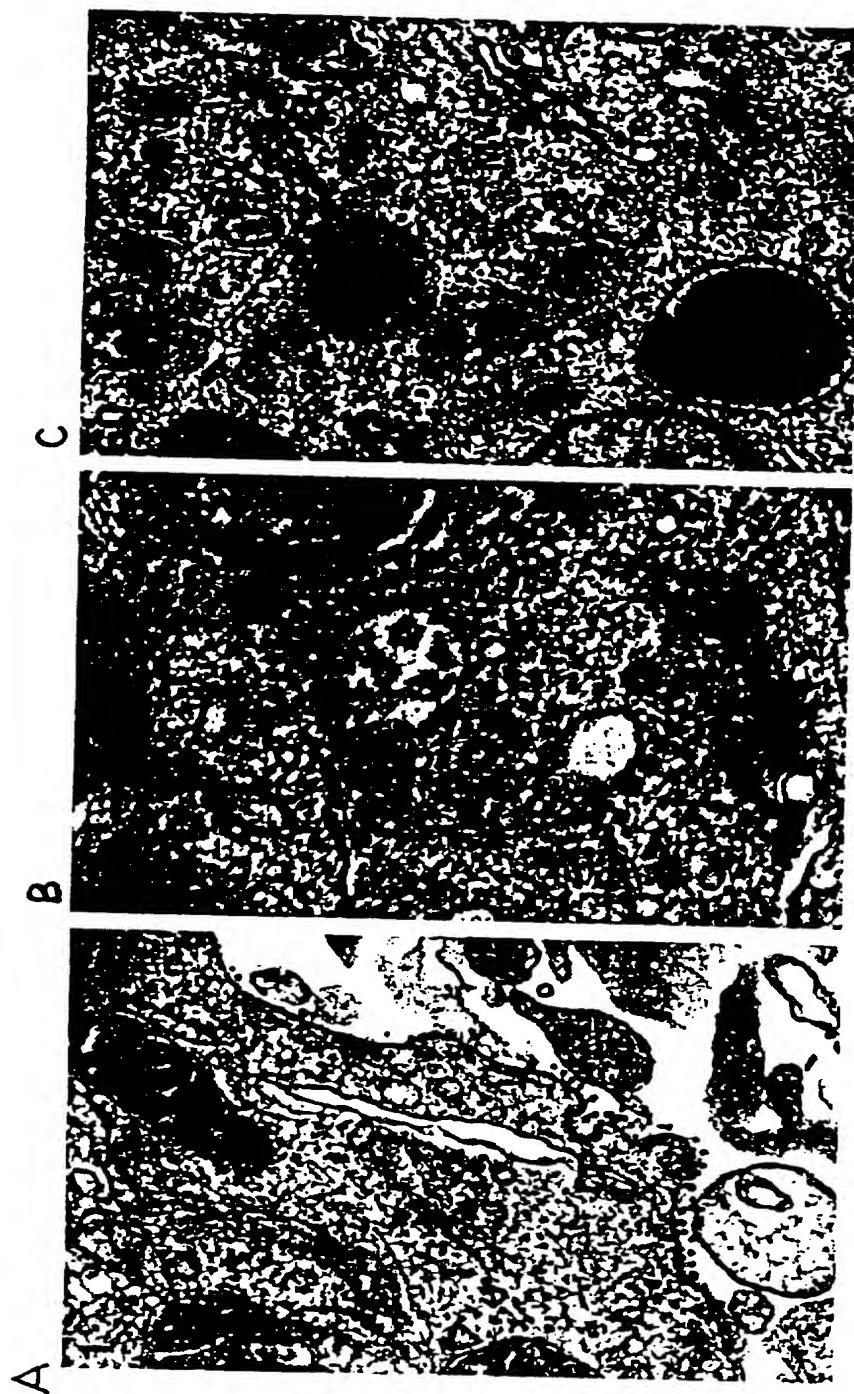


Figure 12

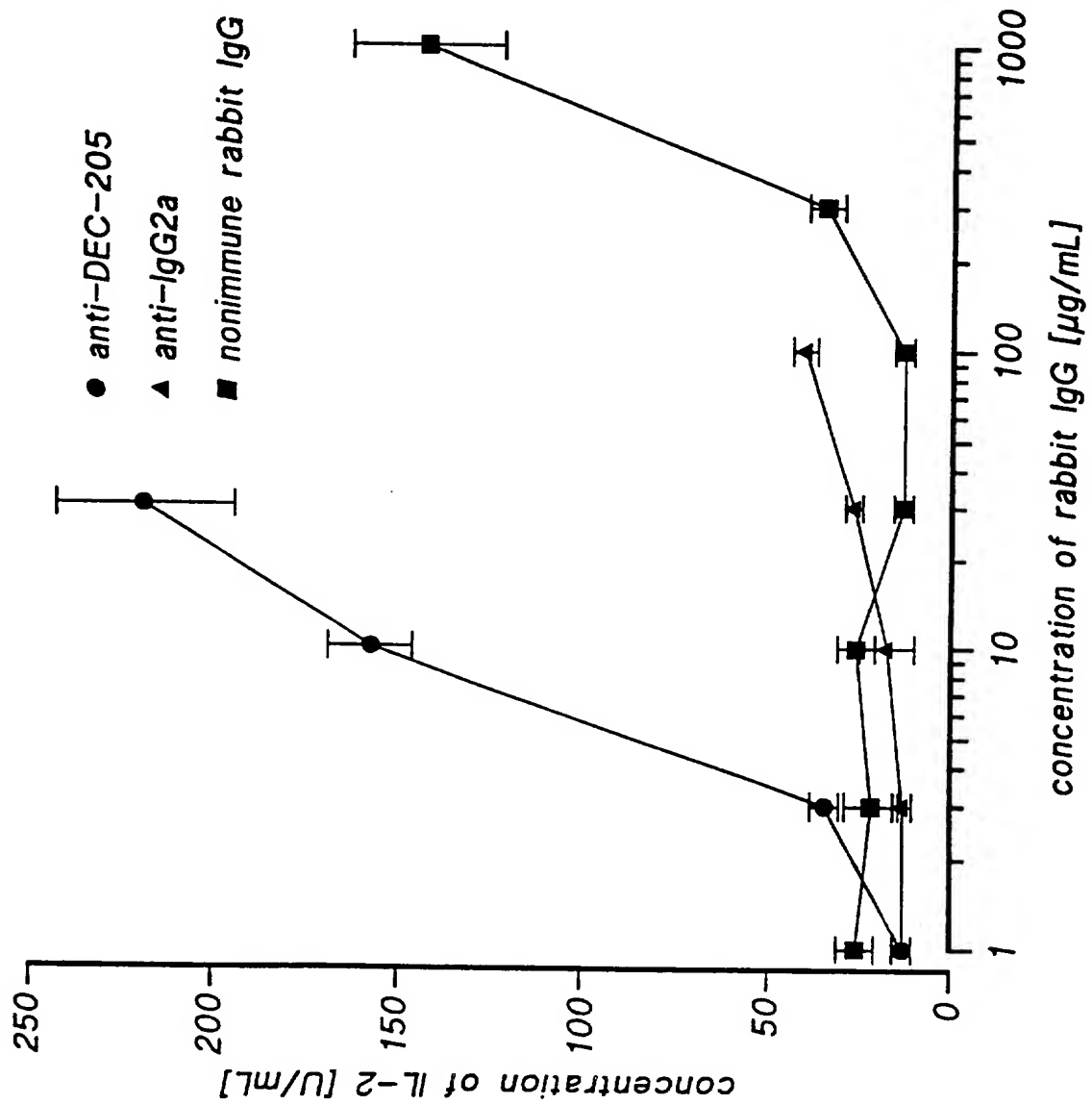
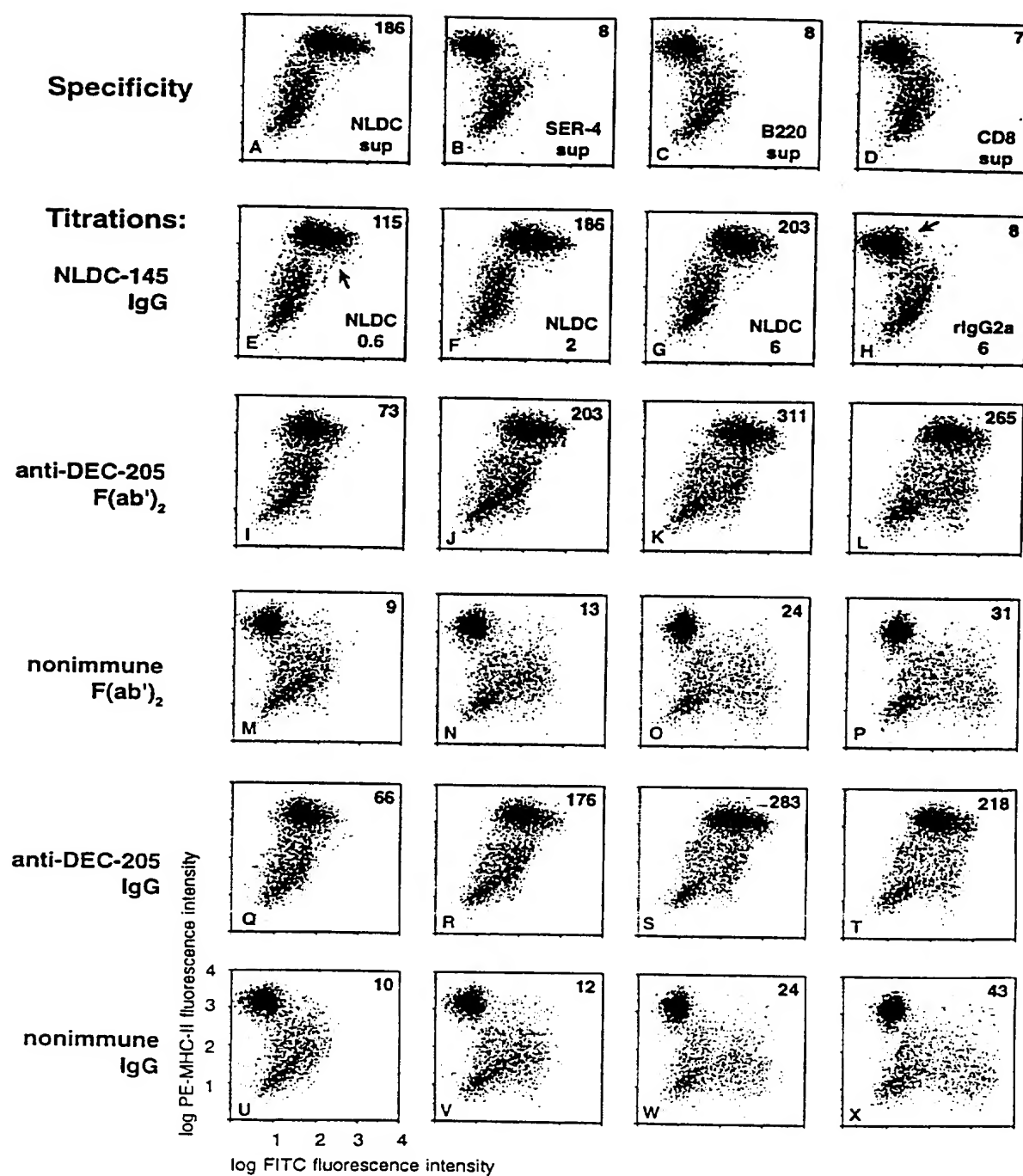


Figure 13



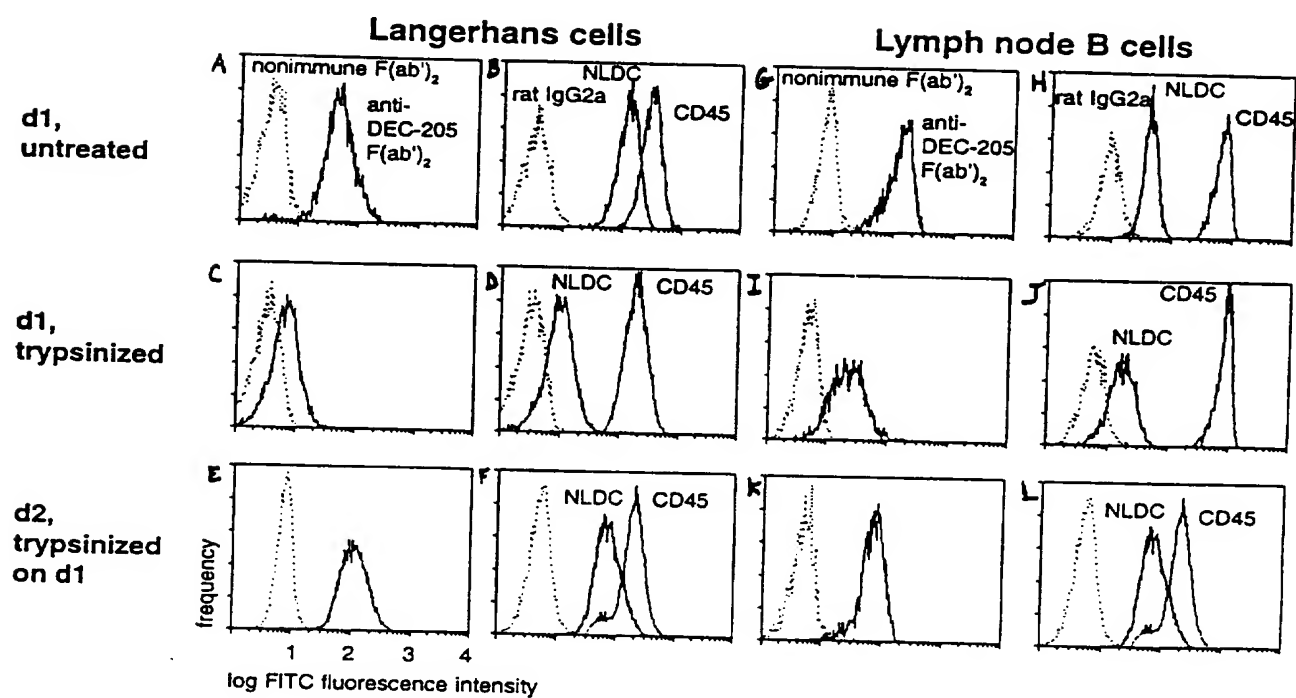


Figure 15

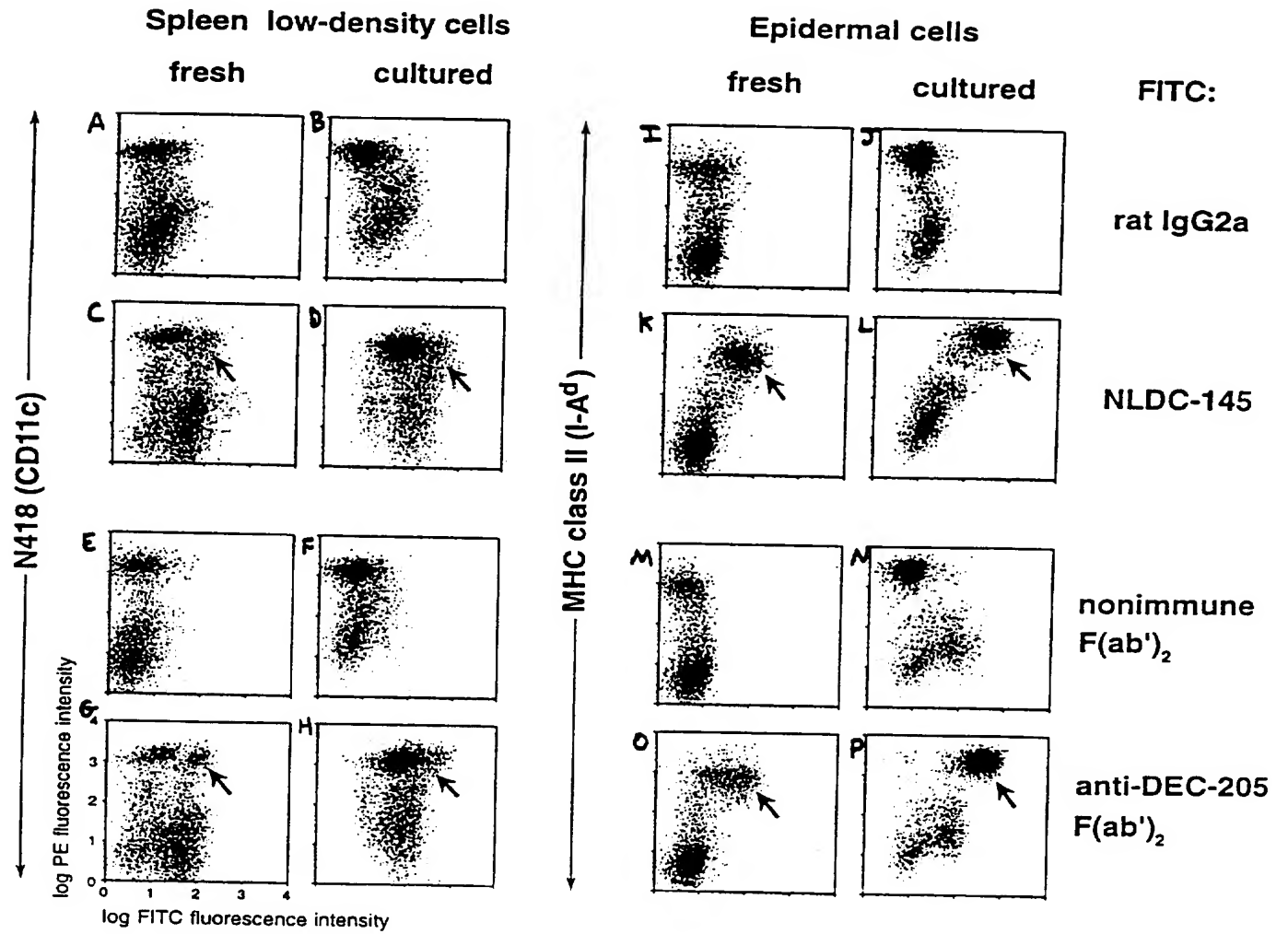


Figure 16

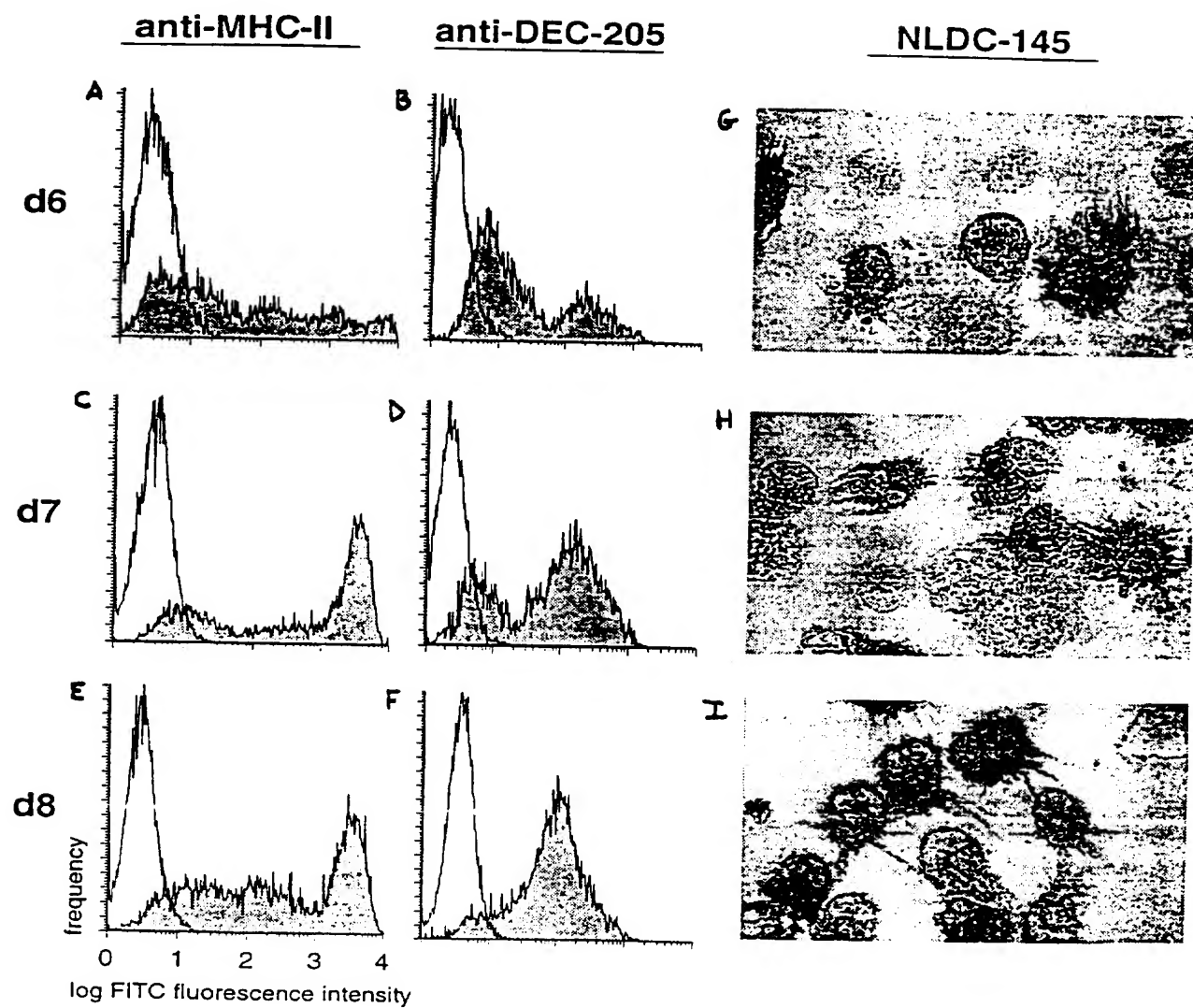


Figure 17

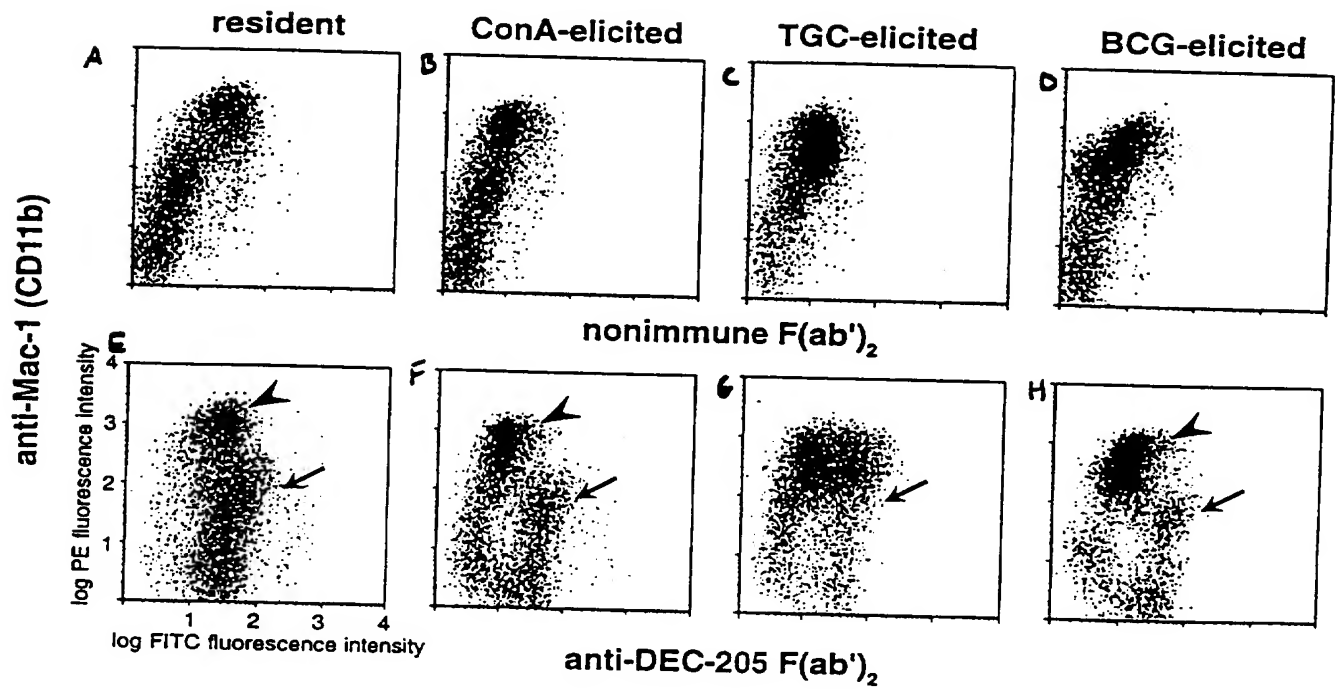


Figure 18

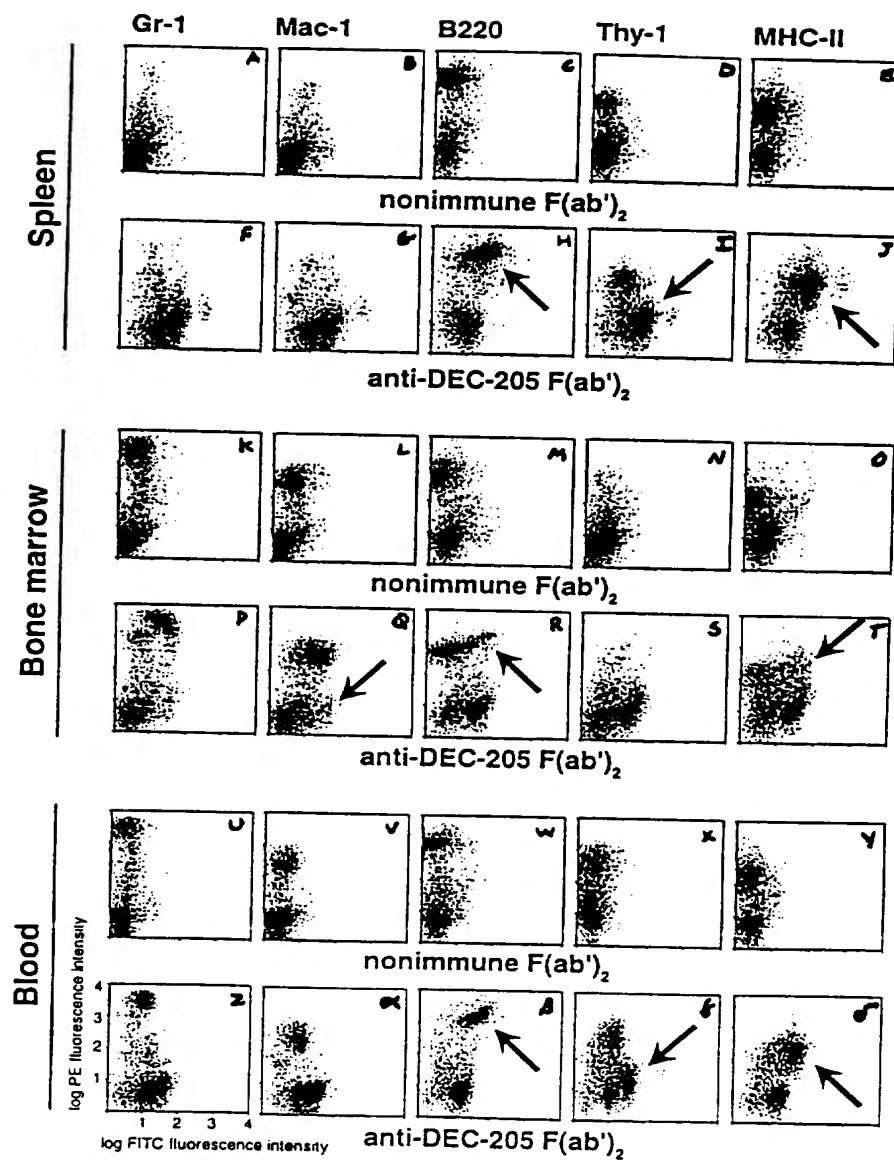


Figure 19

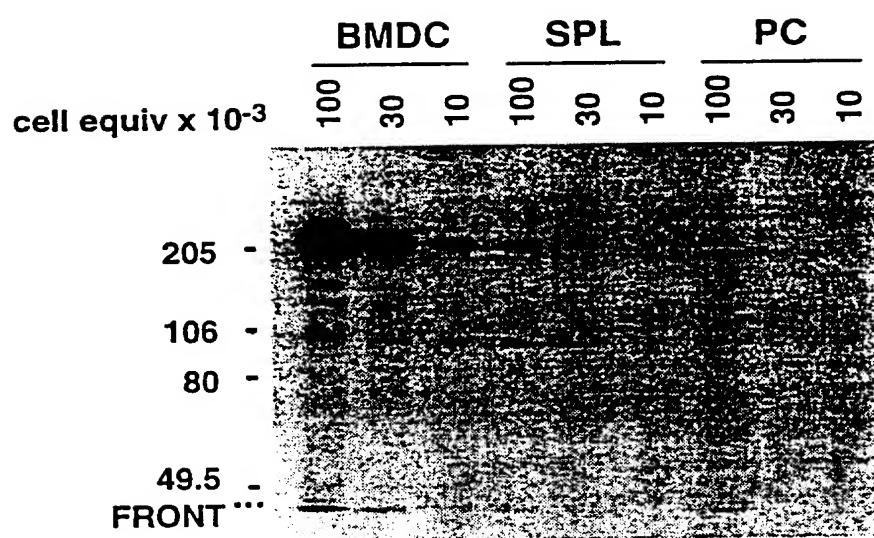
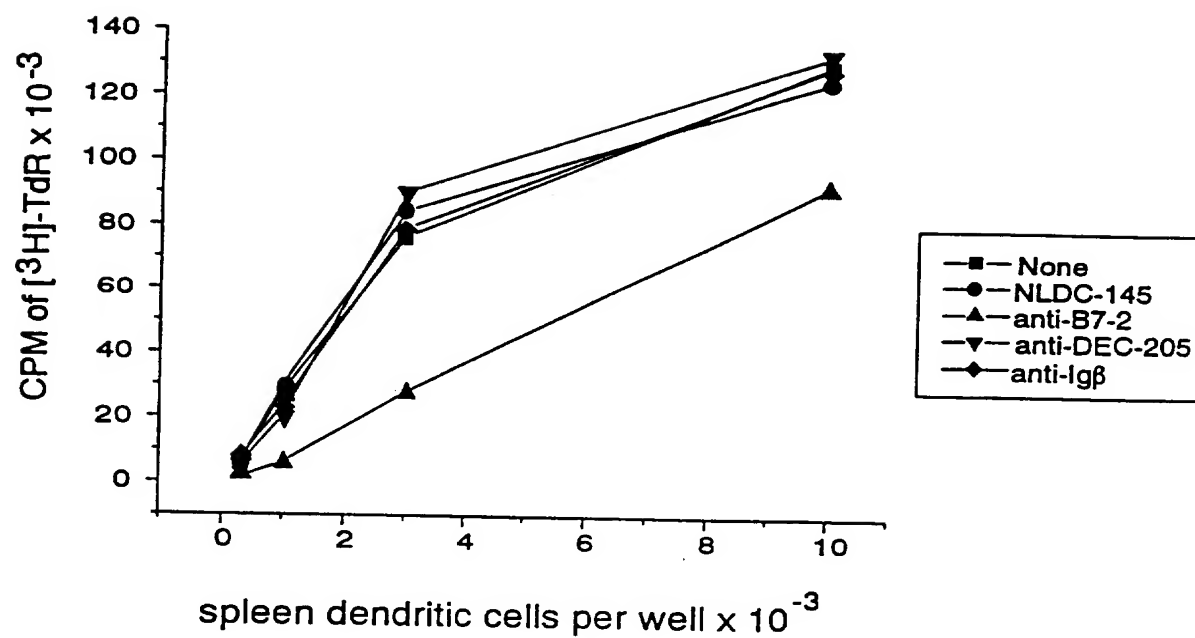


Figure 20



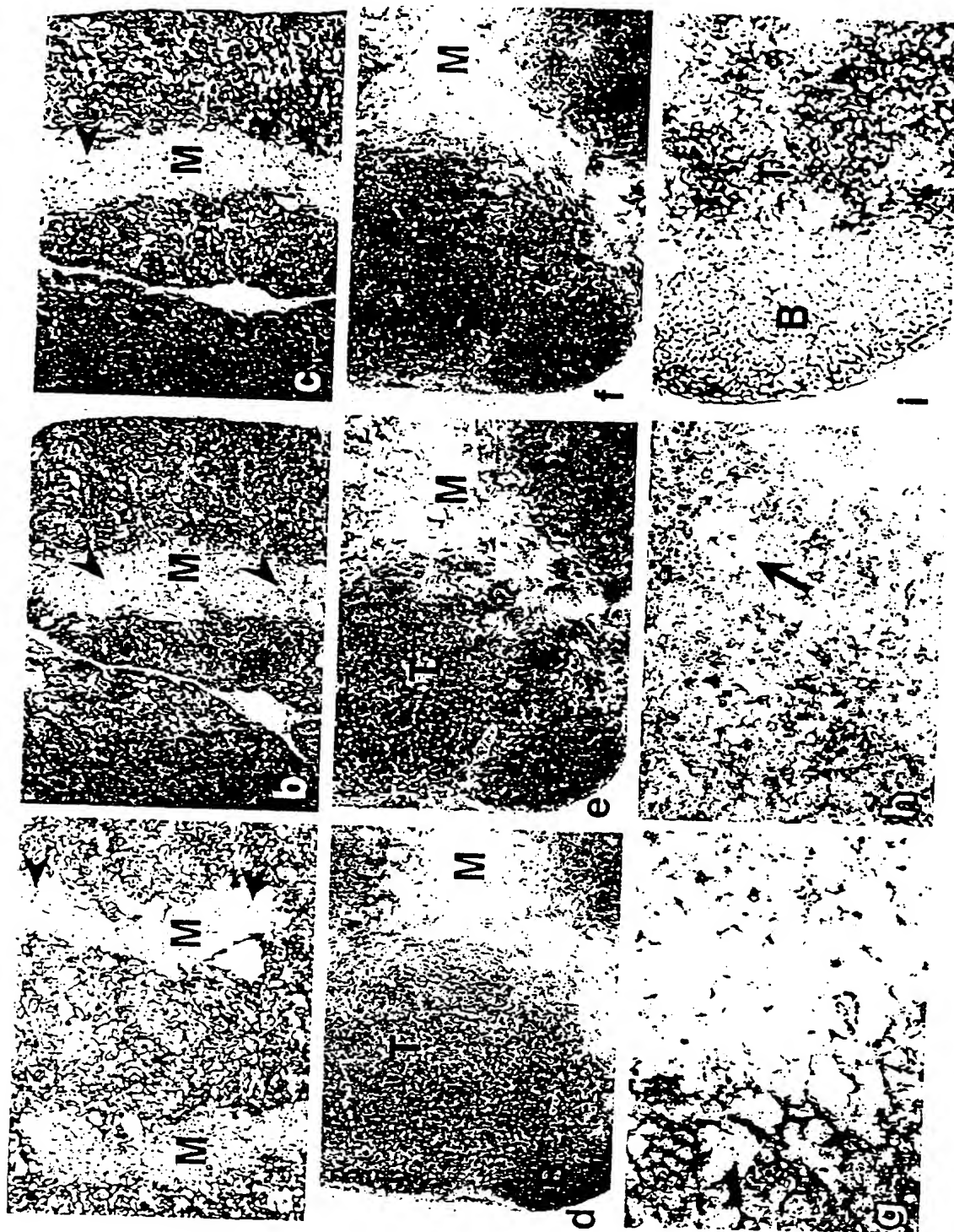


Figure 22

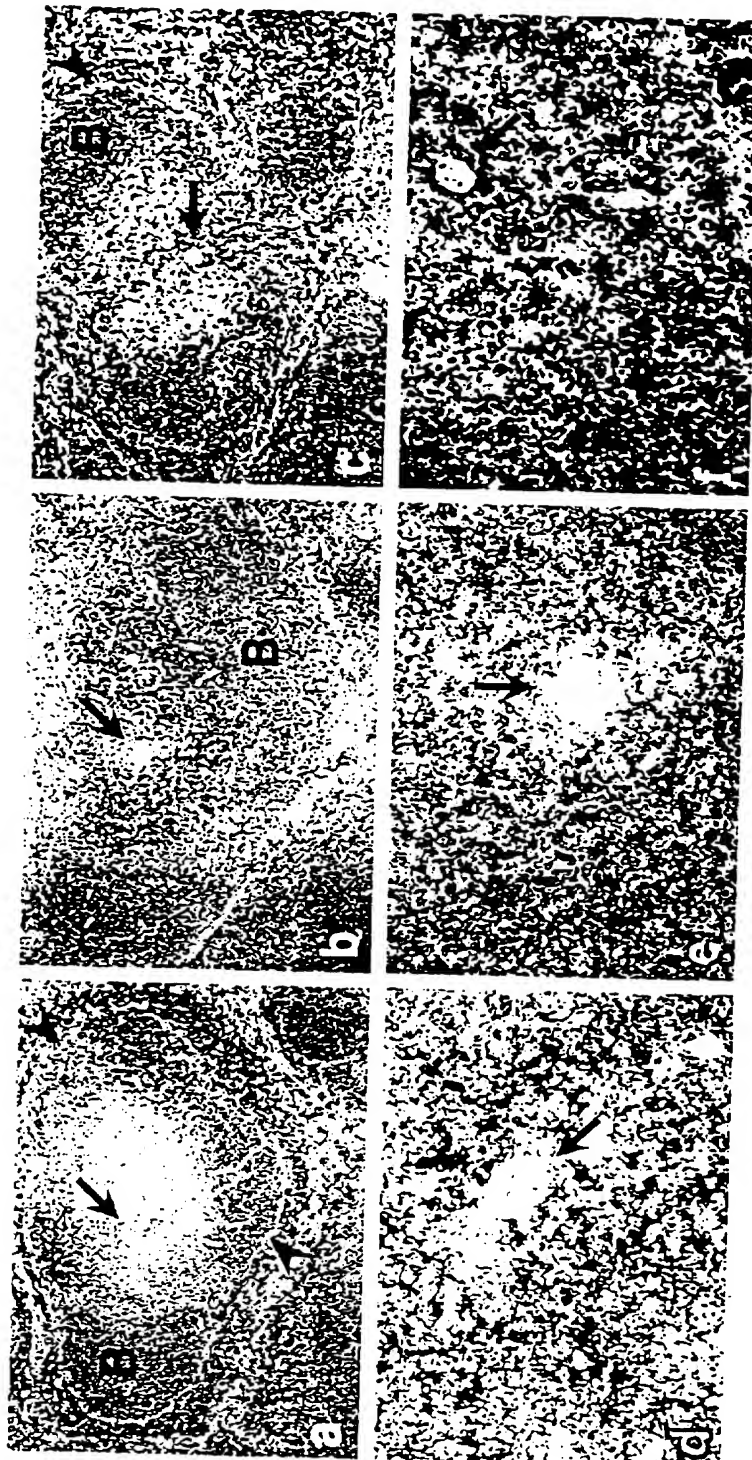
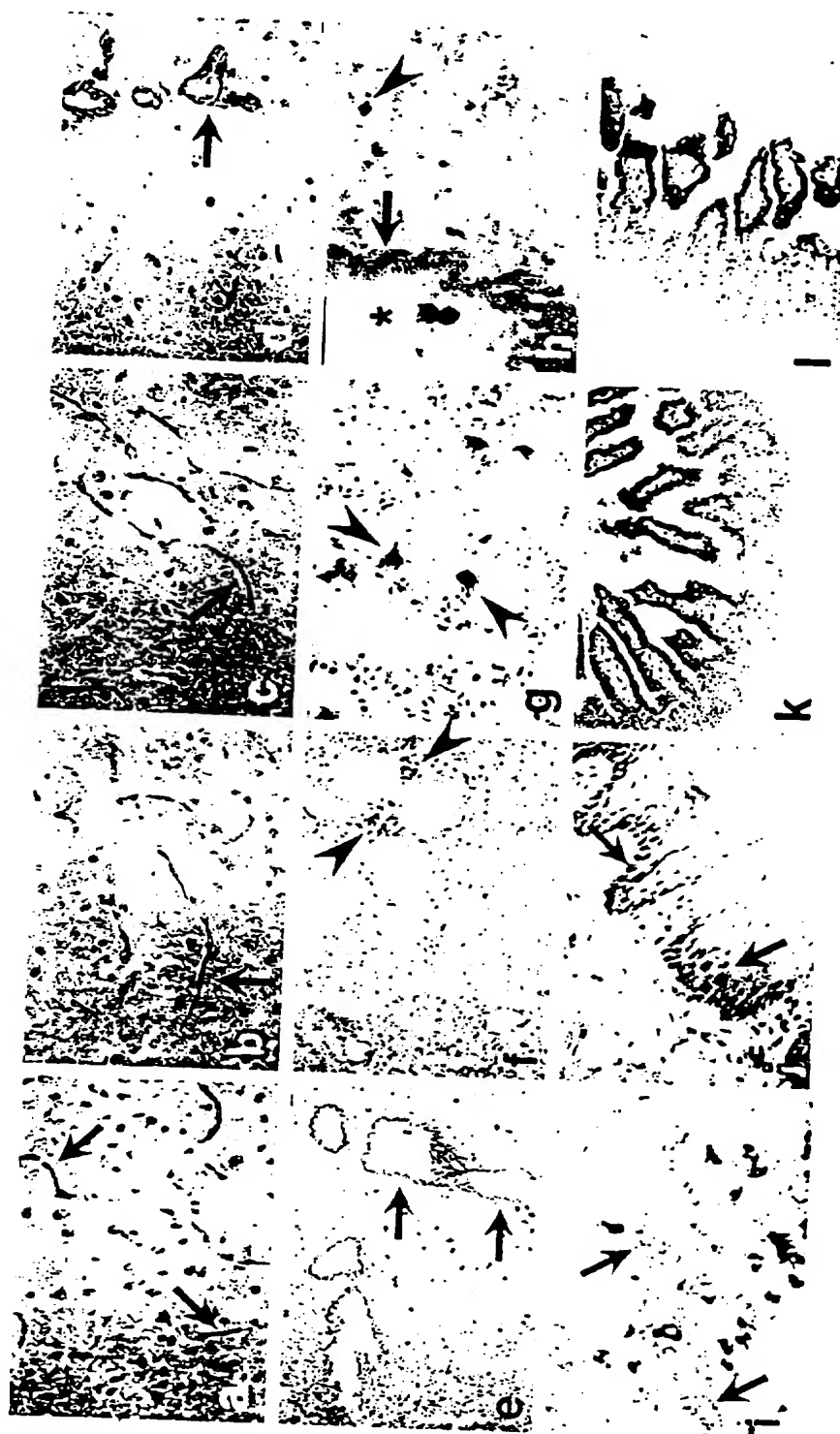


Figure 23



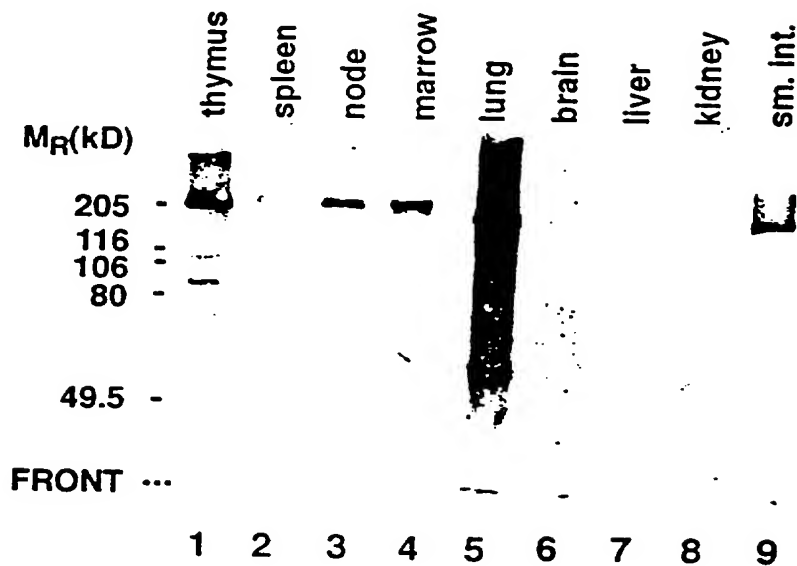
A**B**

Figure 25

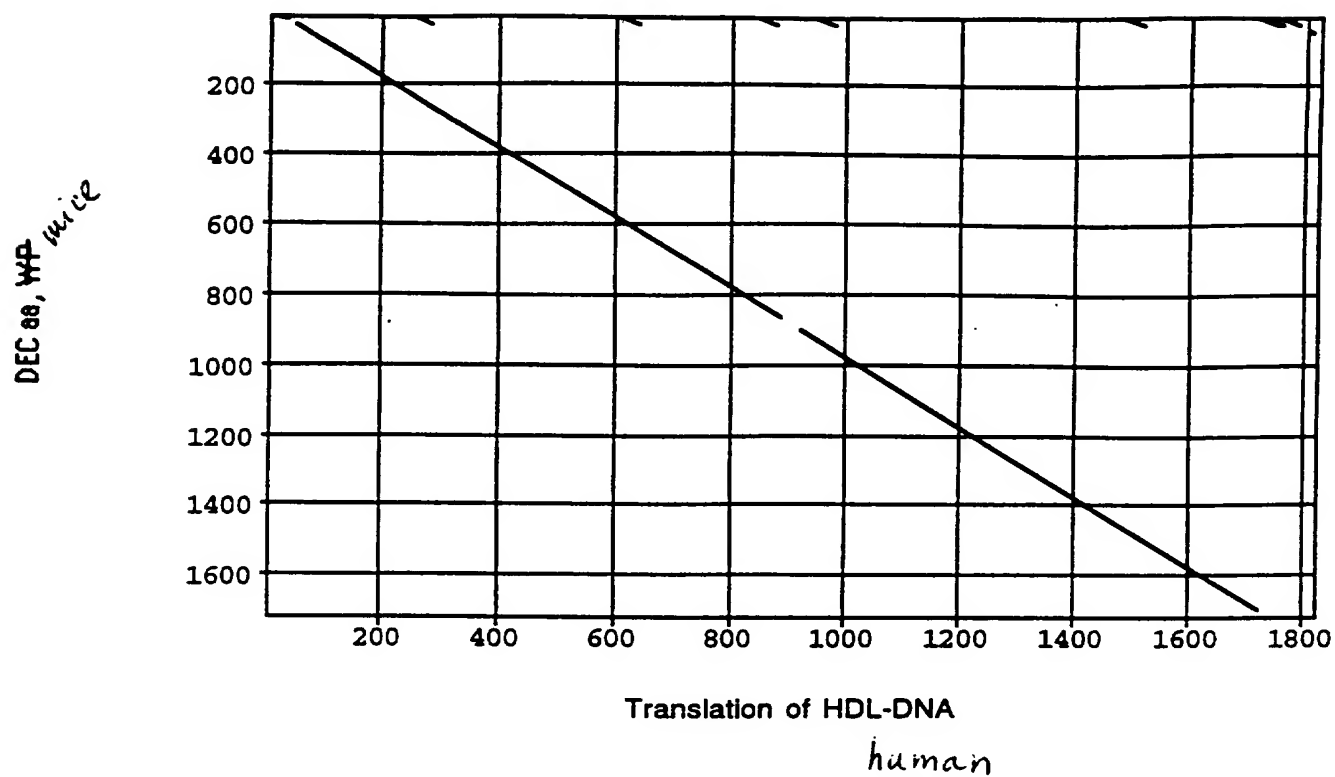
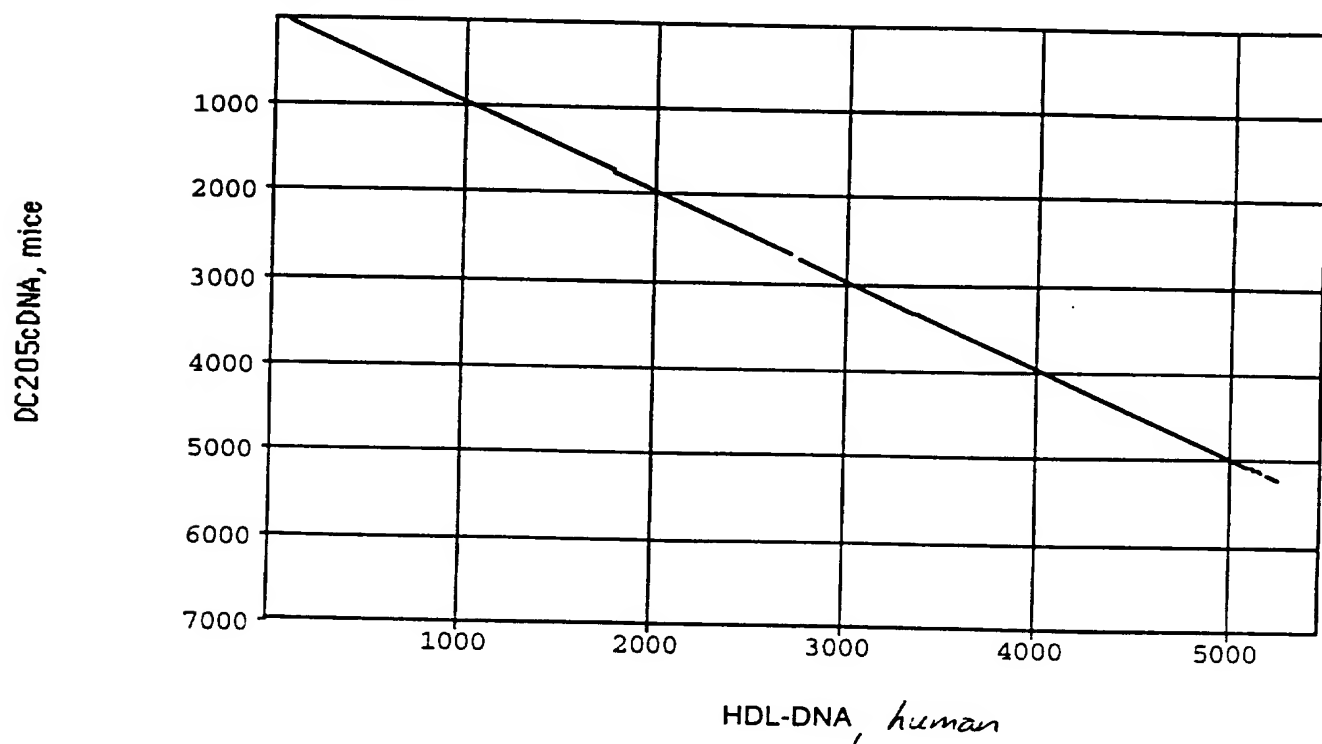


FIGURE 25B



INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/01383

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 A61K38/17 A61K39/00
G01N33/68 A61K47/48 A61K45/00 //A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NATURE (LONDON) 375 (6527). 1995. 151-155. ISSN: 0028-0836, XP000571400 JIANG W ET AL: "The receptor DEC-205 expressed by dendritic cells and thymic epithelial cells is involved in antigen processing." see the whole document ---	1-34
P,A	24TH ANNUAL MEETING OF THE INTERNATIONAL SOCIETY FOR EXPERIMENTAL HEMATOLOGY, DUESSELDORF, GERMANY, AUGUST 27-31, 1995. EXPERIMENTAL HEMATOLOGY (CHARLOTTESVILLE) 23 (8). 1995. 793. ISSN: 0301-472X, XP000571299 STEINMAN R M: "The dendritic cell system of antigen presenting cells." see the whole document --- -/--	1-34

☒ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "&" document member of the same patent family

Date of the actual completion of the international search

4 June 1996

Date of mailing of the international search report

12.06.96

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Fax (+31-70) 340-3016

Authorized officer

Hix, R

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CELLULAR IMMUNOLOGY 163 (1). 1995. 148-156. ISSN: 0008-8749, XP000571286 INABA K ET AL: "Tissue distribution of the DEC-205 protein that is detected by the monoclonal antibody NLDC-145. I. Expression on dendritic cells and other subsets of mouse leukocytes." see the whole document ---	1-34
P,X	CELLULAR IMMUNOLOGY 165 (2). 1995. 302-311. ISSN: 0008-8749, XP000571285 SWIGGARD W J ET AL: "DEC - 205, a 205-kDa protein abundant on mouse dendritic cells and thymic epithelium that is detected by the monoclonal antibody NLDC-145: Purification, characterization, and N-terminal amino acid sequence." see the whole document ---	1-34
P,A	NÉPHROLOGIE, vol. 16, no. 5, 1995, page 381 XP000571265 HAYMANN: "Le récepteur DEC-205 exprimé par les cellules dendritiques et les cellules épithéliales thymiques est impliqué dans l'apprêtement de l'antigène." see the whole document ---	1-34
P,A	(1995) 234 PP. AVAIL.: UNIV. MICROFILMS INT., ORDER NO.: DA9539520 FROM: DISS. ABSTR. INT., B 1995, 56(7), 3684, 1995, XP002004097 SWIGGARD, WILLIAM J.: "Dec - 205, a novel endocytic receptor abundant on mouse dendritic cells and thymic epithelium, has ten C-type lectin domains and mediates antigen presentation" see the whole document ---	1-34
A	J. EXP MED. , vol. 163, April 1986, pages 981-997, XP000571684 G. KRAAL ET AL.: "Langerhans' cells, veiled cells, and interdigitating cells in the mouse recognized by a monoclonal antibody." cited in the application see the whole document -----	1-34